# Commercial Scale Production of Nanomedicines

Edited by
**Aliasgar Shahiwala, Ajay J. Khopade, and Vandana B. Patravale**

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The commercial production of nanomedicines involves challenges related to scalability, reproducibility, safety, and regulatory approvals. It is essential to have access to a preparation method that can produce large quantities of nanomedicines in a scalable way while maintaining a consistently high level of quality and batch-to-batch reproducibility. This book provides comprehensive guidance on the commercial-scale production of nanotechnology-based products, facilities requirements, and quality assurance.

- Authored by industrial and academic experts
- Offers regulatory guidance on the chemistry, manufacturing, and controls (CMC) of nanoparticles and commercial translation of nanomedicines from the lab to market
- Features case studies of successfully marketed nanotechnologies

This book serves as a valuable resource for students and researchers in pharmaceutics, pharmaceutical technology, drug delivery, drug targeting, and biomedical engineering.

It also supports industrial personnel working in nanomedicine manufacturing with the latest updates and those seeking to switch or extend their current product range to nanomedicines or start a new venture in nanomedicine production.

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# Preface

Nanomedicine, an interdisciplinary field at the crossroads of nanotechnology and medicine, has emerged as a transformative approach to addressing some of the most pressing challenges in healthcare. Over the past two decades, the research and development of nanomedicines have progressed significantly, offering innovative solutions in drug delivery, diagnostics, and therapeutic interventions. Despite these advancements, the journey from laboratory-scale research to commercial-scale production has proven to be fraught with challenges. This book, *Commercial Scale Production of Nanomedicines*, aims to bridge the knowledge gap and serve as a comprehensive guide for academia, industry professionals, and regulatory bodies navigating this complex landscape.

The impetus for developing this book stems from the need to address the critical barriers impeding the commercialization of nanomedicines. While extensive research efforts have led to the development of promising nanoparticle-based therapeutics, the scalability, manufacturability, and regulatory compliance of these innovations remain significant hurdles. This book brings together the expertise of leading researchers, industry veterans, and regulatory experts to provide readers with practical insights into overcoming these challenges.

The book comprises 12 meticulously curated chapters, each addressing a pivotal aspect of nanomedicine production. Chapter 1 sets the stage by exploring the overarching challenges associated with the commercial translation of nanomedicines, highlighting the complexities of scaling up from laboratory to production scale. Chapter 2 delves into the regulatory landscape, offering guidance on the chemistry, manufacturing, and controls (CMC) requirements for nanoparticles, which are critical considerations for ensuring product safety and efficacy.

Infrastructure plays a fundamental role in nanomedicine manufacturing, and Chapter 3 examines the infrastructural requirements necessary to meet industrial standards and regulatory expectations. Chapters 4 and 5 focus on the synthesis and functionalization of nanostructures, with discussions on bottom-up and top-down approaches, as well as stealthing techniques such as PEGylation, which are vital for enhancing nanoparticle stability and biocompatibility.

Industry perspectives are interwoven throughout the book, with Chapter 6 providing an in-depth look at the production of lipid nanoparticles for mRNA therapeutics and vaccines—a technology brought to the forefront during the COVID-19 pandemic. Chapter 7 offers a comprehensive analysis of the commercial production of liposomes, emphasizing advancements in manufacturing processes, quality assurance, and control. Similarly, Chapters 8 and 9 address the large-scale production of polymer-based nanoparticles and nanocrystals, respectively, presenting strategies for achieving consistency and scalability.

Inorganic and metallic nanoparticles, with their unique properties and diverse applications, are the focus of Chapter 10, which provides insights into their large-scale production. Chapter 11 discusses the scale-up of nano and microemulsions, highlighting their utility in drug delivery and formulation science. Finally, Chapter 12

explores the emerging field of nanocoated medical devices, underscoring the importance of nanotechnology in enhancing the functionality and performance of medical implants and tools.

This book is not merely a collection of technical information; it is a concerted effort to integrate scientific rigor with practical applicability. By addressing the full spectrum of commercial-scale production—from synthesis to regulatory considerations—we aim to equip readers with the knowledge and tools necessary to advance the field of nanomedicine. The editors—Aliasgar Shahiwala, Ajay J. Khopade, and Vandana B. Patravale—have brought together a diverse group of contributors from industry and academia whose collective expertise ensures the book's relevance and depth.

We hope this volume serves as an invaluable resource for researchers, industrial scientists, and policymakers striving to transform nanomedicine innovations into tangible healthcare solutions. As the field continues to evolve, the insights and frameworks presented in this book will undoubtedly play a crucial role in shaping the future of nanomedicine production and its impact on global health.

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# 1 Challenges for Commercial Translation of Nanomedicines

## *From Lab Scale to Production Scale*

*Ajay J. Khopade and Malay Shah*

### 1.1 INTRODUCTION

Since the emergence of nanotechnology in the 1980s with the discovery of fullerenes [1–3], it has been virtually infused into all sectors of the global economy, benefiting various scientific disciplines [4, 5]. One sector that has gained global attention is nanomedicine, which aims to improve patient convenience, increases the efficiency of treatments, and reduces the toxicity of medicines. This field has garnered the interest of many scientists due to its unique properties that address the problems difficult to solve. Nanoscale medicines, sensors, implants, and imaging modalities have revolutionized the healthcare sector [6–9]. A notable example of this revolution is the recent development and approval of COVID-19 vaccines based on mRNA-lipid nanoparticles [10–12]. This achievement underscores the unprecedented attention paid to the bench-to-bedside translation of nanomedicines worldwide. The approval of COVID-19 vaccines has fostered significant interest in the application of nanomedicine to global healthcare problems. Nanomedicines hold considerable promise for both clinicians and patients by reducing side effects, reducing dosing frequency, improving efficacy, enhancing diagnosis, offering personalized interventions, and enabling the use of highly effective drugs that might otherwise be too toxic for conventional use. As of July 2024, ~200 clinical trials were either ongoing or planned, as indicated by searches using the term "nanoparticle" on the ClinicalTrials.gov website (https://clinicaltrials.gov/). The global nanomedicine market has experienced substantial growth, reaching a value of US$174.20 billion in 2022, poised to grow from US$191.27 billion in 2023 to US$404.08 billion by 2031 [13]. The enthusiasm and high hopes surrounding nanomedicine emerged nearly four decades ago, largely fueled by promising preclinical studies conducted in the 1980s [14, 15]. During this period, research reports highlighted the potential of certain nano-sized macromolecules, such as nanoparticle drug carriers, to passively accumulate in solid tumors. This phenomenon was described as the enhanced permeability and retention (EPR) effect and it is related to specific

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pathophysiological patterns in tumor tissues. This discovery was ground breaking at the time, as it suggested the possibility of selectively targeting tumor tissues while sparing normal tissues [16, 17]. It raised optimism about the development of a tailored therapeutic approach for tumor targeting, often referred to as a "magic bullet." The representative list of nanomedicine approved by global regulatory authorities and currently being commercialized is summarized in Table 1.1.

Nanomedicines exhibit intriguing biological features owing to their nanoscale size, specific structure, and distinct surface properties. These features endow them with numerous advantages, such as improving drug solubility and stability, increasing drug selectivity, modulating controllable drug release in a sustained or responsive manner, synergistic delivery of multiple drugs, elevating bioavailability, enhancing therapeutic effects, and reducing adverse effects. Clinical outcomes have demonstrated that nanomedicines reduce the systemic toxicity and adverse effects of therapeutic agents by altering their pharmacokinetics and biodistributions [71–74]. The development of nanomedicines typically involves reformulating existing drug molecules that are already approved for clinical use, with the exception of nanoparticle-based gene delivery. This approach is logical because enhancing the efficacy or safety of a known molecule is less complex than creating a nanomedicine with an entirely new molecule with an unknown safety and efficacy profile. This strategy also facilitates the quick positioning of nanomedicines at the top of the treatment hierarchy over existing therapies.

Nanomedicines are primarily classified based on the type and structure of their carriers. These categories include liposomes, antibody-drug conjugates, inorganic nanoparticles, lipid nanoparticles, polymer nanoparticles, dendrimers, micelles, polymer-drug conjugates, virus-derived vectors, nanocrystals, cell-derived carriers, and protein-bound nanoparticles [75–77]. Recently, lipid-based nanomedicines have provided valuable platforms for various therapeutics, ranging from liposomal drugs used in clinics to cationic lipid nanoparticles that enable mRNA vaccine delivery. Consequently, lipid-based research will continue to be a hot spot in nanomedicine translation. Polymeric nanomedicines are particularly advantageous due to their ability to solubilize hydrophobic drugs, release drugs over extended periods, reduce drug toxicity, and enhance drug accumulation in tumors [78]. These features make them a promising option for improving the delivery and efficacy of therapeutic agents in cancer and other diseases.

A considerable body of literature showcases the successful applications and utilization of nanomedicine, accompanied by substantial investment influx. However, only a limited number of products have translated into FDA approval and commercialization. Despite numerous studies highlighting the distinct advantages of nanomedicines compared to traditional medicines, the clinical translation of this technology has encountered significant challenges. It is evident that while nanomedicine presents a rapidly expanding market with promising benefits and risks, it also grapples with a degree of uncertainty. This uncertainty may stem from the exaggerated portrayal of early-stage developments. Often, startups, academia, and industry overemphasize basic research findings or initial preclinical discoveries as revolutionary, leading to the exaggeration of potential benefits or the perception of imminent application, despite existing bottlenecks in commercial translation [79].

**TABLE 1.1**
**Representative List of Nanomedicine Approved and Marketed Globally**

| Trade Name | Nanoplatform | Drug | Application | Company | Approval Agency/Year | Reference |
|---|---|---|---|---|---|---|
| Abelcet® | Lipid complex | Amphotericin B | Antifungal | Liposome Company, Inc | USFDA (1995) | [18] |
| Abraxane® | Albumin nanoparticle | Paclitaxel | Anticancer | Bristol-Myers Squibb | USFDA (2005) | [19] |
| Adagen® | Polymer-protein conjugate | Adenosine deaminase | For the treatment of severe combined immunodeficiency disease (SCID) associated with a deficiency of adenosine deaminase | Enzon Pharmaceuticals, Inc | USFDA (1990) | [20] |
| Adynovate® | Polymer-protein conjugate | Recombinant antihemophilic factor | For the treatment of Hemophilia A | Baxalta, Inc | USFDA (2015) | [21] |
| AmBiosome® | Liposome | Amphotericin B | Antifungal | NeXstar Pharmaceuticals | USFDA (1997) | [22] |
| Apealea® | Micelle | Paclitaxel | Anticancer | Oasmia Pharmaceutical AB | EMA (2018) | [23] |
| Aristada | Nanocrystal | Aripiprazole luroxil | For the treatment of schizophrenia | Alkermes, Inc | USFDA (2015) | [24] |
| Avinza® | Nanocrystal | Morphine sulfate | For the management of chronic pain | Pfizer, Inc | USFDA (2002) | [25] |
| Bevetex® | Polymer-lipid nanoparticles | Paclitaxel | Anticancer | Sun Pharmaceutical | India (2014) | [26] |
| Cimzia® | Antibody-polymer conjugate | Certolizumab pegol | For the treatment of Crohn's disease | UCB, Inc | USFDA (2008) | [27] |
| Curosurf® | Lipid surfactant | Poractant alfa | For the treatment of respiratory distress syndrome (RDS) | Chiesi Farmaceutici, S.p.A | USFDA (1999) | [28] |
| DaunoXome® | Liposome | Daunorubicin citrate | Anticancer | NeXstar Pharmaceuticals, Inc | USFDA (1996) | [29] |
| Depocyt® | Liposome | Cytarabine | Anticancer | Pacira Pharmaceuticals | USFDA (1999) | [30] |
| DepoDur® | Liposome | Morphine sulfate | Postoperative analgesia | Endo Pharmaceuticals | USFDA (2004) | [31] |
| Dexferrum® | Iron nanoparticles | Iron dextran | Iron deficiency in chronic kidney | American Regent, Inc | USFDA (1996) | [32] |

*(Continued)*

**TABLE 1.1** *(Continued)*
**Representative List of Nanomedicine Approved and Marketed Globally**

| Trade Name | Nanoplatform | Drug | Application | Company | Approval Agency/Year | Reference |
|---|---|---|---|---|---|---|
| Diprivan® | Nanoemulsion | Propofol | Anesthetic agent | AstraZeneca LP | USFDA (2001) | [33] |
| Doxil® | Liposome | Doxorubicin hydrochloride | Anticancer | Johnson and Johnson | USFDA (1995) | [34] |
| Emend® | Nanocrystal | Aprepitant | Antiemetic | Merck & Co., Inc | USFDA (2003) | [35] |
| Estrasorb® | Nanoemulsion | Estradiol | Treatment of moderate to severe vasomotor symptoms in postmenopausal women | Novavax, Inc | USFDA (2003) | [36] |
| Focalin® XR | Nanocrystal | Dexmethylphenidate hydrochloride | Attention-deficit hyperactivity disorder in children | Novartis Pharmaceuticals Corporation | USFDA (2008) | [37] |
| Genexol-PM® | Polymeric micelle | Paclitaxel | Anticancer | Samyang Genex Corporation | Korea (2007) | [38] |
| Hensify® | Hafnium oxide nanoparticles | Hafnium oxide | Locally advanced soft tissue sarcoma | Nanobiotix | EMA (2019) | [39] |
| Invega Sustenna® | Nanocrystal | Paliperidone palmitate | Schizophrenia | Janssen Pharmaceuticals, Inc | USFDA (2009) | [40] |
| Inveltys® | Nanosuspension | Loteprednol etabonate | Postoperative ophthalmic inflammation | Kala Pharma | USFDA (2018) | [41] |
| IV Emend® | Nanocrystal | Fosaprepitant dimeglumine | Antiemetic | Merck & Co., Inc | USFDA (2008) | [42] |
| Krystexxa® | Polymer-protein conjugate | Pegloticase | Severe and treatment refractory chronic gout | Savient Pharmaceuticals, Inc | USFDA (2010) | [43] |
| Lipodox® | Liposome | Doxorubicin hydrochloride | Anticancer | Sun Pharmaceutical Industries Ltd. (SPIL) | USFDA (2013) | [44] |
| Macugen® | Polymer-protein conjugate | Pegaptanib sodium | Wet AMD | Pfizer, Inc | USFDA (2004) | [45] |
| Marqibo® | Liposome | Vincristine sulfate | Anticancer | Talon Therapeutics, Inc | USFDA (2012) | [46] |

*(Continued)*

**TABLE 1.1** *(Continued)*
**Representative List of Nanomedicine Approved and Marketed Globally**

| Trade Name | Nanoplatform | Drug | Application | Company | Approval Agency/Year | Reference |
|---|---|---|---|---|---|---|
| Megace®ES | Nanocrystal | Megestrol acetate | Anorexia, cachexia and AIDS-related weight loss | Par Pharmaceutical | USFDA (2005) | [47] |
| Mepact® | Liposome | Mifamurtide | Anticancer | Takeda France SAS | EMA (2009) | [48] |
| Mircera® | Polymer-protein conjugate | Methoxy polyethylene glycol-epoetin beta | For anemia associated with chronic kidney disease | Hoffman La-Roche | USFDA (2007) | [49] |
| Moderna Vaccine | Lipid nanoparticle | mRNA vaccine | Prevention of COVID-19 infection | Moderna, Inc | USFDA (2020) | [50] |
| Myocet® | Liposome | Doxorubicin hydrochloride | Anticancer | Teva Pharmaceutical | EMA (2000) | [51] |
| NanOss® | Nanocrystal | Hydroxyapatite granules | Bone substitute | RTI Surgical, Inc | USFDA (2005) | [52] |
| Neulasta® | Polymer-protein conjugate | Pegfilgrastim | Neutropenia | Amgen, Inc | USFDA (2002) | [53] |
| Oncaspar® | Polymer-protein conjugate | Pegaspargase | acute lymphoblastic leukemia (ALL) | Enzon Pharmaceuticals, Inc | USFDA (2006) | [54] |
| Onivyde® | Liposome | Irinotecan hydrochloride | Anticancer | Merrimack Pharmaceuticals | USFDA (2015) | [55] |
| Onpattro® | Liposome | Patisiran sodium | Polyneuropathy | Alnylam Pharmaceuticals, Inc | USFDA (2018) | [56] |
| Ontak® | Engineered fusion protein nanoparticle | Denileukin diftitox | Cutaneous T-cell lymphoma (CTCL) | Eisai Co., Ltd | USFDA (1999) | [57] |
| Pegasys® | Polymer-protein conjugate | PEGylated interferon alpha-2A | Hepatitis B and hepatitis C | Genentech USA, Inc | USFDA (2002) | [58] |
| PegIntron® | Micelle | PEGylated interferon alpha-2b | Chronic hepatitis C | Merck & Co. Inc | USFDA (2001) | [59] |

*(Continued)*

**TABLE 1.1** ***(Continued)***
**Representative List of Nanomedicine Approved and Marketed Globally**

| Trade Name | Nanoplatform | Drug | Application | Company | Approval Agency/Year | Reference |
|---|---|---|---|---|---|---|
| Pfizer-BioNTech vaccine | Lipid nanoparticle | mRNA vaccine | Prevention of COVID-19 infection | Pfizer, Inc | USFDA (2020) | [60] |
| Plegridy® | Polymer-protein conjugate | Peginterferon beta-1a | Relapsing remitting multiple sclerosis in adults | Biogen, Inc | USFDA (2014) | [61] |
| Rebinyn® | Polymer-protein conjugate | Recombinant coagulation factor IX | Hemophilia B | Novo Nordisk, Inc | USFDA (2017) | [62] |
| Restasis® | Nanoemulsion | Cyclosporine | Chronic dry eye | Allergan, Inc | USFDA (2003) | [63] |
| Ritalin LA® | Nanocrystal | Methylphenidate hydrochloride | Attention-deficit hyperactivity disorder in children | Novartis Pharmaceuticals Corporation | USFDA (2002) | [64] |
| Somavert® | Polymer-protein conjugate | Pegvisomant | For acromegaly | Pfizer, Inc | USFDA (2003) | [65] |
| Tricor® | Nanocrystal | Fenofibrate | For hypercholesterolemia | AbbVie, Inc | USFDA (2004) | [66] |
| Venofer® | Iron nanoparticles | Iron sucrose | Iron deficiency in chronic kidney disease | American Regent, Inc | USFDA (2000) | [67] |
| Visudyne® | Liposome | Verteporfin | For the treatment of choroidal neovascularization due to age-related macular degeneration | QLT, Inc | USFDA (2002) | [68] |
| Zanaflex® | Nanocrystal | Tizanidine hydrochloride | For the management of spasticity | Acorda Therapeutics, Inc | USFDA (2002) | [69] |
| Zilretta® | Microsphere | Triamcinolone acetonide | For the treatment of osteoarthritis | Flexion Therapeutics, Inc | USFDA (2017) | [70] |

A crucial gap exists between basic sciences (preclinical research) and clinical sciences (clinical research), necessitating concerted efforts to bridge this translational divide. As a scientific community, it is imperative to adopt a different perspective and celebrate the success of emerging modalities such as monoclonal antibodies, siRNA, mRNA, and CRISPR technology [80–83]. The COVID vaccine exemplifies the culmination of decades of fundamental learning and substantial investment in mRNA-based formulations of lipid nanoparticles [7–9]. Today, we find ourselves in an era where a select few approved nanomedicines are being safely and effectively delivered to millions of patients worldwide. These successes underscore the importance of perseverance and long-term investment in nanomedicine. They also highlight the need for realistic expectations and transparent communication about the progress and challenges in the field. By continuing to support rigorous research and fostering collaboration between basic and clinical sciences, the potential of nanomedicine can be more fully realized, leading to innovative treatments and improved patient outcomes.

It is widely agreed upon that the process of developing nanomedicines is characterized by prolonged timelines, high costs, and a significant risk of failure at each developmental stage. While advancements over the past two decades may have somewhat alleviated the challenges associated with nanomedicine development, various barriers in manufacturing, biological and preclinical studies, regulatory policies, intellectual property (IP), and governmental regulations—still impede its transition from laboratory research to clinical applications. Addressing these challenges is crucial for the successful clinical translation and commercialization of nanomedicine. The present review discusses the challenges, perspectives, and pathways crucial to the clinical translation and commercialization of nanomedicine (Figure 1.1). These include the following:

1. **Manufacturing Challenges**
   - **Lack of Precise Control over Manufacturing Parameters:** Ensuring consistency in nanoparticle/nanomaterial formation is challenging.
   - **Scalability and Batch-to-Batch Variability**: Technical challenges arise in scaling up production while maintaining product consistency.
   - **Specialized Manufacturing Infrastructure**: Expensive and technology-specific infrastructure is needed.
   - **GMP Compliance**: Ensuring good manufacturing practice (GMP) compliance is critical yet challenging.
   - **Reproducibility Issues**: Controlling size distribution, mass, and stability (aggregation, degradation) is difficult.
   - **High Costs**: Specialized raw materials and infrastructure are expensive.
   - **Need for Skilled Workforce**: Outstanding researchers and skilled workers are essential.
2. **Challenges in Characterization of Nanomaterials**
   - Need of outstanding researchers and skilled workers.
3. **Challenges about Preclinical Animal Studies**
   - **Inadequate Preclinical Model:** Inadequate preclinical models that accurately mimic human disease conditions.

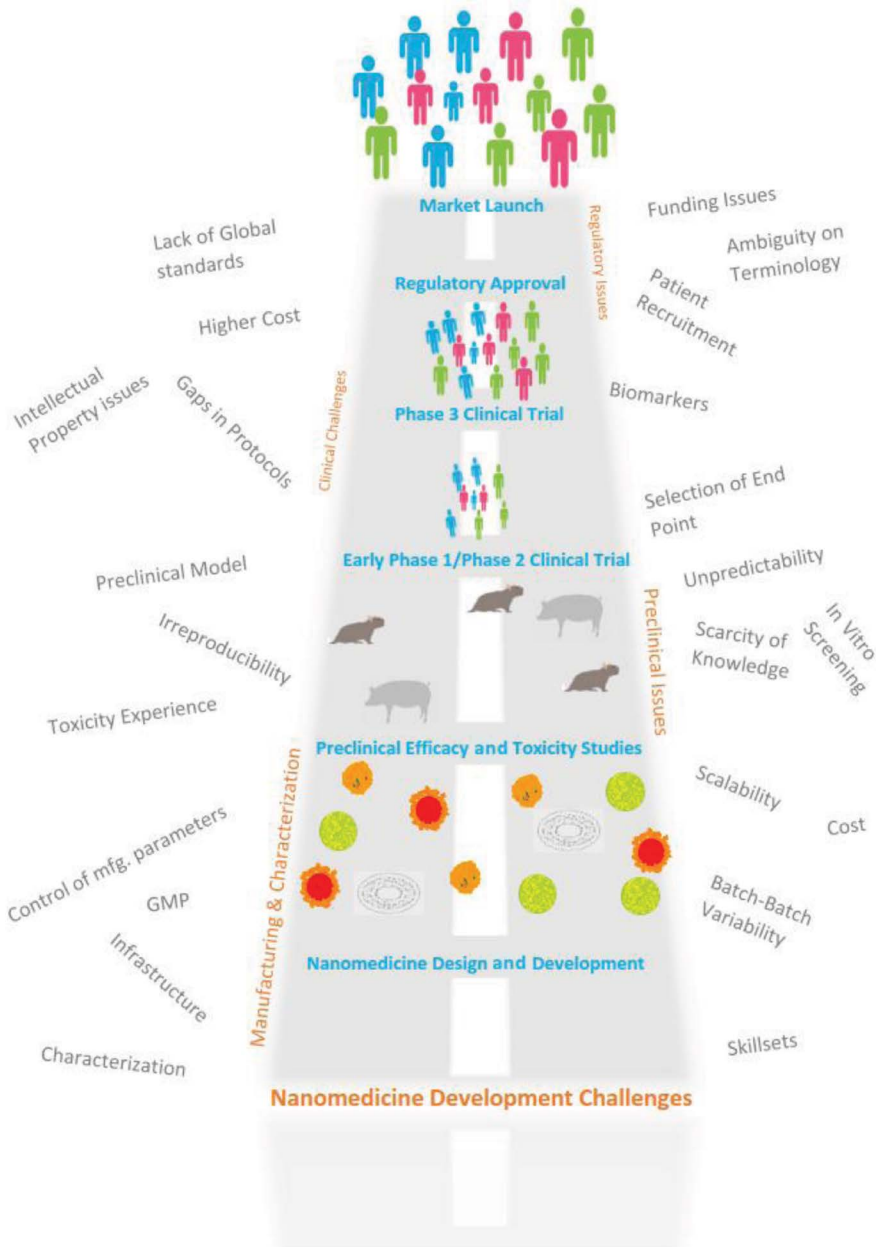


**FIGURE 1.1** Nanomedicine development challenges.

- **Irreproducibility of Preclinical Data**: Inconsistent preclinical research data hampers progress.
- **Scarcity of Knowledge on Nanomaterial Behavior**: Limited understanding of how nanomaterials interact with cells and tissues.
- **Lack of Rational Characterization Strategies**: Need for comprehensive preclinical characterization via multiple techniques.
- **Limited Knowledge of Biocompatibility and Biodistributions**: Insufficient data on how nanomaterials behave in biological systems.
- **Limited Experience with Toxicity Assessment**: Nanomaterials require new toxicity assessment methods.
- **Lack of Standard *In Vitro* Screening Platforms**: Need for *in vitro* platforms that correlate well with *in vivo* performance.
- **Unpredictable Toxicity and ADME**: Unique physicochemical properties of nanomaterials lead to unpredictable absorption, distribution, metabolism, and excretion.

4. **Clinical Trial Challenges**
   - **High Costs and Lengthy Trials**: Clinical trials for nanomedicines are expensive and time-consuming.
   - **Challenges in Patient Recruitment**: Strict inclusion/exclusion criteria and ethics committee delays complicate recruitment.
   - **Lack of Consensus on Procedures and Protocols**: Inconsistent methodologies impact trial design and outcomes.
   - **Clinical End Point:** Identifying clinical end point aligned to the differentiating properties of nanomedicine
   - **Superiority of Nanomedicine:** Establishing superiority of nanomedicine over existing treatment regimen.
5. **Regulatory Challenges**
   - **Ambiguity in Terminology**: Confusion over terms like "nanomaterials," "nanomedicines," and "nanoscale."
   - **Lack of Global Consensus**: No standard classification for nanotechnology-based products.
   - **Lack of Technical Specifications and Standards**: Absence of clear guidelines and best practices.
   - **Regulatory Bodies' Caution**: Lack of experience and expertise leads to a precautionary stance.
   - **Country-Specific Policies**: Variations in regulations across countries pose challenges for international clinical trials.
6. **Intellectual Property**
   - Complexity of nanomedicine formulations can make patenting difficult, requiring a strategic approach to IP management.
   - **Complex IP Protection**: Involvement of multiple stakeholders complicates IP issues.
   - **Emerging Patent Thickets**: Overlapping patent claims create legal and strategic challenges.
   - **Loss of Patent Shelf Life**: Long development timelines reduce the effective patent life.

7. **Funding Challenges**
    - **Investor Perception**: Perceived low return on investment.
    - **Prolonged Development Pathways**: Deter funders due to high risk and long timelines.
    - **Lack of Experienced Investors**: Difficulty in gauging the potential for successful translation.
    - **Reluctance of Big Pharma**: Hesitancy to invest in early-stage preclinical research.
    - **Lack of Industry Support**: Challenges in securing funds for first-in-human (FIH) trials. Limited potential to reach FIH trials without strong industry backing.
    - **Inadequate Due Diligence**: Insufficient review of translational potential during funding processes.
    - **Lack of Financial Incentives**: Difficulty attracting long-term private and public investments.
8. **Other Factors**
    - **Media Hype and Misinformation**: Overhyped technology creates false hopes and misinformed public perception.
    - **Focus on Publications**: Overemphasis on academic publications rather than practical technology transformation.
    - **Lack of Interdisciplinary Communication**: Need for better collaboration between industry, academia, and regulatory bodies.
    - **Business Expertise Deficit**: Universities and research institutes often lack the business acumen to commercialize technology.
    - **Wide Gap between Academia, Industry, and Regulatory Bodies**: Bridging this gap is crucial for successful translation and commercialization.

Despite these challenges, the potential benefits of nanomedicine make it a worthwhile pursuit. Continued efforts to address these barriers through collaborative research, innovation in analytical and manufacturing technologies, and a clear regulatory framework will help in advancing the field and bringing more nanomedicines to market.

## 1.2 MANUFACTURING CHALLENGES IN CLINICAL TRANSLATION

One of the foremost challenges in the clinical translation of nanomedicine is an appropriate understanding of its chemistry, manufacturing, and quality control (CMC). The complexity inherent in manufacturing nanomedicines is a critical factor that influences their rapid and effective transition to commercialization. Key challenges include large-scale manufacturing, controlled and reproducible production, product quality and stability, raw material costs and the need for specialized infrastructure, equipment, and skillsets [84–87]. Many nanomedicines fail to meet these requirements and, consequently, do not reach the market. Hence, it is imperative to assess the feasibility of the developed nanomedicine at an early stage of its development. The manufacturing process of nanomedicine involves both top-down

and bottom-up approaches, employing methods such as sonication, homogenization, milling, and crystallization [74]. While these techniques are manageable at a small scale, they pose significant challenges when scaled up for large-scale production. Understanding manufacturing feasibility during the early stages not only prevents the need for product redevelopment but also saves time, cost, and resources required to recreate preclinical data for submission. Furthermore, reformulating the product may impact the established proof of concept in animal models. The complexity of nanomedicine manufacturing necessitates meticulous oversight to minimize batch-to-batch variations. A key distinction between nanomedicine and conventional drug products lies in their *in vivo* performance. Variations in bioavailability, biodistribution, drug concentrations at the target site, and off-target sites result in notable differences in efficacy and toxicity compared to conventional drugs [88, 89]. These differences stem from the distinct physicochemical properties of nanomedicine, including particle size, polydispersity, surface morphology, drug encapsulation, and release. Ensuring the quality attributes of these properties is crucial, preferably established early in the development process, ideally before preclinical testing in animal models. The complexity of nanomedicine is further compounded when attempting to incorporate multifunctionality. For instance, surface modification of nanomedicine with ligands specific to targets or creating nanomedicine with active drug-release properties triggered by internal or external stimuli (such as pH, temperature, enzymes, light, and ultrasound) [90, 91]. Integrating these multiple components into a single system inevitably leads to complexity and increased costs in large-scale production. The focus of nanomedicine development should prioritize addressing unmet clinical needs rather than blindly pursuing versatility. Therefore, the design of nanomedicines should be streamlined to ensure efficient and reproducible large-scale manufacturing while meeting clinical requirements.

Achieving controlled and reproducible manufacturing of nanomedicine at a reasonable cost is essential. It is vital to take into account the overall costs involved in developing and scaling up of nanomedicine manufacturing. There is always uncertainty surrounding the success of nanomedicine development compared to the significant investment required for the process. Hence, it is crucial to thoroughly assess the investment risk, profit margins, and the benefit-to-risk ratio of the proposed nanomedicine in comparison to other developmental projects. Moreover, when devising investment strategies for nanomedicine development and subsequent clinical applications, the expenses related to manufacturing equipment, instrumentation, and facilities must also be considered. It is imperative to have clarity on these aspects before proceeding to clinical trials. Given the inherent complexity and expense associated with developed nanomedicine, it should offer substantial value to the pharmaceutical industry to justify funding its development pathways [92].

Before embarking on the development of nanomedicine, it is crucial to assess the potential benefits it offers, whether it be improvements in efficacy, safety, or patient convenience. The clearer these benefits are, the more easily a significant price premium can be justified once the product reaches the market. Additionally, the size of the patient population is another key consideration [88]. A larger patient population

translates to greater sales and market potential for the product. However, it is essential to weigh these factors against the lower cost of traditional medicines offered by comparator products. For instance, if the comparator product is a simple oral tablet and the nanomedicine requires intravenous administration, stakeholders must evaluate whether physicians and patients are willing to transition from a daily oral routine to a parenteral treatment. While the latter may be less frequently required, it typically entails administration in an outpatient clinic and comes with a significantly higher total treatment cost. Considering these factors, the commercial viability of nanomedicine is unlikely unless there is clear potential for improved efficacy or safety outcomes. It is imperative to address these commercial feasibility questions from the outset of a nanomedicine development program, before committing resources to its development.

Another crucial aspect concerns the safeguarding of IP associated with the technology [93–95]. The protection of IP, including patents, holds significant importance in any commercialization endeavor. Securing patents for inventions provides inventors with the opportunity to recoup investments by restricting competitors from entering the market, fostering innovation, generating revenues, identifying whitespace opportunities, and avoiding legal issues such as infringement. Without adequate patent protection, the developed nanomedicine may struggle to attract investors willing to finance further development and obtain the premium it merits. Obtaining IP protection can be particularly complex, especially in cases involving new chemical entities, targeting ligands, antibodies, and other elements that entail the involvement of multiple stakeholders. Additionally, caution must be exercised regarding "patent thickets" in the design of nanomedicine, as these can lead to costly litigation and impede overall commercialization efforts. To mitigate the risk of litigation, freedom-to-operate (FTO) assessments, also known as "product clearance" or "right to use" opinions, should be conducted well in advance, preferably during the early stages of preclinical or prototype evaluation studies. FTO exercises aid in securing patents by comprehensively understanding publicly available technologies and correlating them with one's own invention. They also provide an opportunity to modify prototypes if they infringe upon existing patents. Understanding the patent process, the patent landscape, and identifying whitespace opportunities are essential for translational research and the development of innovations for clinical use.

### 1.2.1 Key Manufacturing Challenges

1. **Large-Scale Manufacturing**
   - **Scalability**: Processes like sonication, homogenization, milling, and crystallization are manageable at small scales but become challenging when scaled up. The transition from laboratory-scale to industrial-scale production can introduce variability and affect the consistency and quality of the nanomedicine.
2. **Controlled and Reproducible Manufacturing**
   - **Batch-to-Batch Variability**: Ensuring consistency across batches is crucial. Variations in particle size, polydispersity, zeta potential,

surface morphology, and drug encapsulation from batch to batch can lead to differences in bioavailability, biodistribution, and efficacy.
    - **Quality by Design (QbD)**: Implementing QbD principles during the early stages of development helps define critical quality attributes (CQAs) such as particle size, polydispersity, drug encapsulation, and release profiles. These attributes need to be well defined before preclinical testing in animal models.
3. **Stability of the Product**
    - **Physicochemical Stability**: Nanomedicines must maintain stability during manufacturing, storage, and administration. Issues like aggregation and degradation can compromise their effectiveness and safety.
4. **Cost of Raw Materials and Infrastructure**
    - **High Costs of Raw Materials and Infrastructure**: The raw materials and specialized infrastructure required for nanomedicine manufacturing are often expensive. Additionally, the need for specialized equipment and skilled personnel further increases costs.
    - **GMP Compliance**: Establishing a manufacturing facility for nanomedicine that complies with GMP standards is necessary but costly and complex.
5. **Skillsets**
    - **Specialized Expertise**: The production of nanomedicines requires a multidisciplinary team with expertise in nanotechnology, chemistry, biology, and engineering. Training and retaining such a skilled workforce is challenging.

## 1.3 KEY CONSIDERATIONS FOR NANOMEDICINE DEVELOPMENT

### Early-Stage Considerations

- **Feasibility and Practicability**: Assessing the manufacturing feasibility at the early stages of development is crucial. This assessment helps avoid redevelopment of the product, saving time, costs, and resources.
- **Scalability**: Potential scalability issues should be identified early to avoid later-stage complications. Understanding the challenges associated with scaling up manufacturing processes can prevent delays and additional costs.

### Multifunctionality and Complexity in Technology

- **Surface Modification and Stimuli-Responsive Systems**: Incorporating target-specific ligands or active drug-releasing properties adds complexity and cost.
- **Simplification**: Focus should be on addressing unmet clinical needs with simple, reproducible designs to ensure efficient large-scale manufacturing.

### Cost Considerations

- **Investment Risk**: High costs and the uncertainty of success require careful analysis of the investment risk, profit margins, and benefit-to-risk ratio.

- **Manufacturing Costs**: Consider costs for equipment, instrumentation, and facilities. These must be clear before proceeding to clinical trials to ensure the pharmaceutical industry's willingness to fund development.

### Quality Control and Standardization

- **Defining Key Quality Attributes**: CQAs must be identified and standardized early in the development process. These attributes are essential for ensuring consistent performance and safety.
- **Minimizing Batch Variations**: Close scrutiny of the manufacturing process is required to minimize batch-to-batch variations. Standardizing production protocols and implementing stringent quality control measures are necessary for reproducibility.

### Degree of Improvement

- **Efficacy and Safety**: The primary consideration is the extent of improvement in efficacy or safety that nanomedicine offers compared to traditional treatments. Significant improvements justify a higher price premium.
- **Patient Convenience**: Ease of administration plays a crucial role. For instance, transitioning from an oral tablet to an intravenous treatment requires clear benefits to outweigh the convenience of the existing method.

### Patient Population Size

- **Market Potential**: The larger the patient population, the greater the potential market and sales. However, the therapeutic or administrative benefits must significantly outweigh the costs of existing treatments to be commercially viable.

### Impacts on Clinical Translation

- **Proof of Concept**: Any changes in the formulation during redevelopment can impact the established proof of concept in animal models. Consistency in formulation and manufacturing processes is essential to maintain the validity of preclinical data.
- **Regulatory Approval**: A comprehensive understanding of CMC aspects is critical for regulatory submissions. Clear and consistent manufacturing protocols, coupled with robust quality control measures, facilitate the approval process.

### IP Protection

- **Patent Protection**: Securing patents is essential for recovering investments, promoting innovation, generating revenue, and avoiding legal issues like infringement.
- **Complexity of IP**: IP protection can be complex in cases involving new biological entities and multiple stakeholders. Simplifying IP strategies can save time and cost.
- **Patent Thickets and Litigation Risks**: Navigating patent thickets can be costly and impede commercialization. Conducting FTO analyses early can

mitigate these risks by identifying potential infringements and allowing for necessary modifications. Performing FTO analyses during early preclinical stages helps secure patents and understands the technology landscape.

Successful nanomedicine development requires addressing several key considerations from the outset. By focusing on these aspects, the potential of nanomedicines can be realized, and the transition of nanomedicine from the lab to the clinic can be more efficient and commercially viable, ultimately leading to innovative treatments that meet clinical needs.

## 1.4 ANALYTICAL TECHNIQUES FOR QUALITY CONTROL MANUFACTURE

Thorough characterization of nanomedicines is crucial for ensuring their efficacy and safety. Standardized analytical methods and specific characterization strategies are vital for the clinical translation of nanomedicines. Characterization requirements depend on various factors such as the type of nanomedicine, route of administration, indications, and physicochemical properties like particle size, surface charge, and encapsulation efficiency [96–99]. Here are key analytical techniques (Figure 1.2) used for quality control in nanomedicine manufacturing:

All nanomedicines must undergo comprehensive characterization to assess their predefined CQAs, which directly impact their efficacy and safety. The utilization of standardized analytical methods and specific characterization strategies holds significant importance in facilitating the clinical translation of nanomedicines. However, it's essential to recognize that the characterization of nanomedicine depends significantly on factors such as the type of nanomedicine, route of administration, intended indications, and additional physicochemical properties including particle size, surface charge, surface area, surface reactivity, shape, core-shell structure, hydrophobicity, coating, porosity, crystallinity, as well as the presence of free and encapsulated drug and release kinetics. Due to the inherent complexity of manufacturing processes and the diverse range of materials utilized, the characterization of nanomedicine necessitates distinct analytical approaches tailored to analyze and interpret the physicochemical properties specific to each nanomedicine formulation. For instance, dynamic light scattering (DLS) is commonly employed for accurately determining particle size in nanomedicine samples that are monodisperse, yet achieving monodispersibility in practice can be challenging. In cases where samples exhibit polydispersity, an alternative approach is required, integrating DLS with high-resolution techniques like field flow fractionation (FFF), particle tracking analysis (PTA), or transmission electron microscopy (TEM).

Numerous reports, guidelines, and perspectives on the characterization of nanomedicine or nanomaterials have been issued by various regulatory bodies such as the EMA [100–102] and FDA [103]. Several standards-setting organizations, including the American Society for Testing and Materials (ASTM International) and the International Organization for Standardization (ISO), are actively involved in developing nanotechnology standards [104]. The ISO standard "ISO/TR 18196:2016" offers guidance on commercially available techniques relevant to measuring common

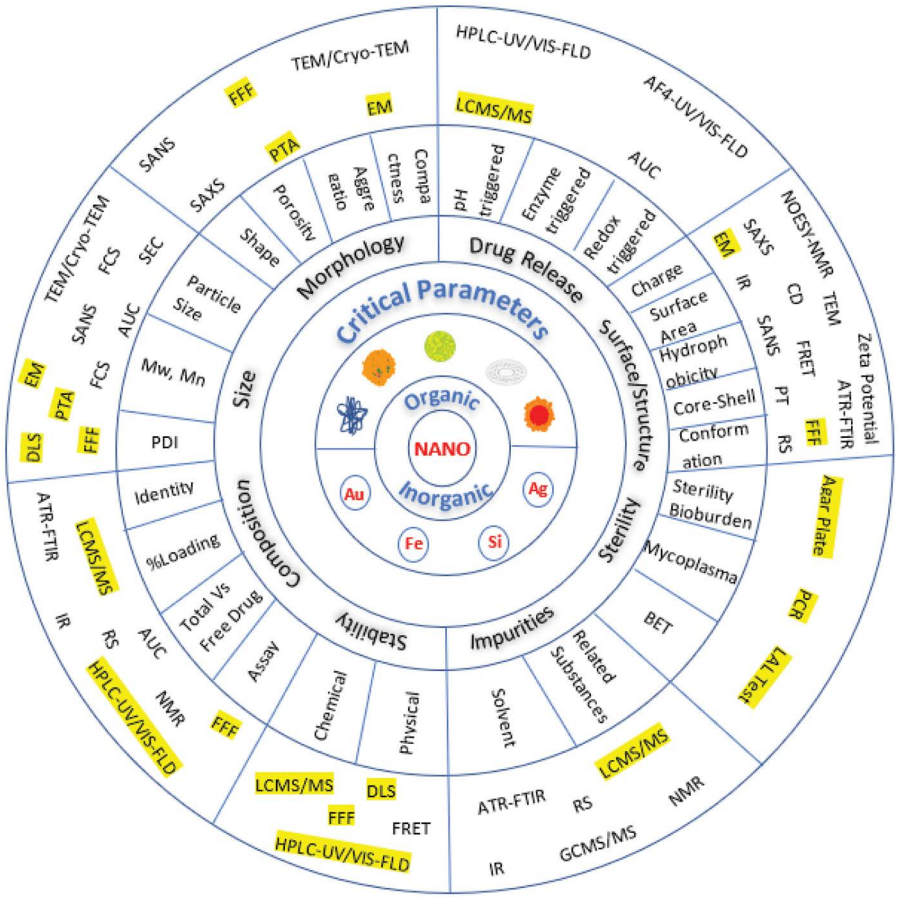


**FIGURE 1.2** Critical parameters of nanomedicines and its analytical techniques for characterization. *Abbreviations*: DLS: dynamic light scattering, PT: potentiometry, FFF: field flow fractionation, LC–MS/MS: liquid chromatography–mass spectrometry, HPLC–UV: high-performance liquid chromatography-ultraviolet, AUC: analytical ultracentrifugation, IR: infrared, FRET: fluorescence resonance energy transfer, CD: circular dichroism, PCR: polymerase chain reaction, AF4-UV: asymmetric field flow fractionation-ultraviolet, Cryo-TEM: cryogenic transmission electron microscopy, FCS: fluorescence correlation spectroscopy, SEC: size-exclusion chromatography, PTA: particle tracking analysis, GC–MS/MS: gas chromatography–mass spectrometry, NMR: nuclear magnetic resonance, RS: remote sensing, Vis-FLD: visible-fluorescence detector, ATR-FTIR: attenuated total refection—Fourier transform infrared spectroscopy, NOESY-NMR: nuclear Overhauser effect spectroscopy-nuclear magnetic resonance, BET: bacterial endotoxin, SANS: small-angle neutron scattering, SAXS: small-angle X-ray scattering, TEM: transmission electron microscopy, EM: Electron microscopy, LAL assay: limulus amebocyte lysate assay, Si: silicon, Fe: iron, Au: gold, Ag: silver. NCL-approved techniques are highlighted.

physicochemical parameters for nanomaterials. ASTM standards for nanotechnology cover a wide range of topics, including nanotechnology terminology, property testing, and health and safety concerns.

Here are key analytical techniques used for quality control in nanomedicine manufacturing:

### 1.4.1 Physicochemical Characterization

#### Particle Size and Distribution

- **DLS**: Widely used for determining particle size in monodisperse samples. However, in polydisperse samples, it should be complemented with other techniques.
- **MALS**: Measures the angular variation in scattered light intensity to determine the size distribution.
- **Laser Diffraction**: Analyses size distribution based on the scattering pattern of a laser beam passing through the sample.
- **FFF**: Separates particles based on size and can be used in combination with DLS for more accurate measurements.
- **TEM**: Provides high-resolution images to measure particle size and morphology.
- **PTA**: Tracks the movement of particles under a microscope to determine size distribution.

#### Surface Properties

- **Zeta Potential Measurement**: Evaluates the surface charge of nanoparticles, which affects stability and interactions with biological systems.
- **X-Ray Photoelectron Spectroscopy (XPS)**: Analyzes surface chemistry and elemental composition.
- **Atomic Force Microscopy (AFM)**: Provides surface topography and mechanical properties at the nanoscale.

#### Structural Characterization

- **TEM and Scanning Electron Microscopy (SEM)**: Offer detailed structural information about particle morphology and core-shell structures.
- **X-Ray Diffraction (XRD)**: Determines crystalline structure and phase purity.
- **Nuclear Magnetic Resonance (NMR)**: Provides information on the chemical environment of atoms within the nanomaterial.

#### Composition and Purity

- **Fourier Transform Infrared Spectroscopy (FTIR)**: Identifies chemical bonds and functional groups.
- **Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**: Measures elemental composition with high sensitivity.
- **High-Performance Liquid Chromatography (HPLC)**: Used for separating and quantifying components within the nanomedicine.

### 1.4.2 Functional Characterization

**Drug Encapsulation and Release**

- **Ultracentrifugation and Dialysis**: Techniques to measure drug encapsulation efficiency.
- ***In Vitro* Release Studies**: Simulate the release profile of the drug from the nanocarriers under physiological conditions.

**Biological Interactions**

- ***In Vitro* Cell Studies**: Assess biocompatibility, cytotoxicity, and cellular uptake using techniques like flow cytometry and confocal microscopy.
- ***In Vivo* Biodistribution Studies**: Use imaging techniques such as positron emission tomography (PET) or magnetic resonance imaging (MRI) to track the distribution of nanoparticles in animal models.

The development and clinical translation of nanomedicines require rigorous and comprehensive characterization using a range of analytical techniques. Understanding the CQAs and employing standardized methods are essential for ensuring the safety, efficacy, and reproducibility of nanomedicines.

## 1.5 TRANSLATING PRECLINICAL EFFICACY TO CLINICAL OUTCOME

Transitioning from animal proof-of-concept data to successful clinical outcomes poses an additional challenge in nanomedicine development. Presently, there is a prevailing tendency in the research community to prioritize positive and noticeable results over the presentation and discussion of technical findings. This trend has led to biased and irreproducible preclinical research. An online survey conducted by Delphine et al. between 2016 and 2017, intended for trainees involved in bench research, indicated that an average of approximately 25% did not seem to receive optimal mentoring. A total of 39.2% revealed having been pressured by a principal investigator or collaborator to produce "positive" data. About 62.8% admitted that the pressure to publish influences how they report data. Participated trainees believe that the pressure to publish affects honest reporting, mostly emanating from our system of rewards and advancement. The publication process itself affects faculty and trainees and appears to influence a shift in their ethics from honest reporting ("negative data") to selective reporting, data falsification, or even fabrication [105]. Another survey conducted by the American Society for Cell Biology in 2015, involving 900 participants, revealed that over two-thirds of respondents had encountered situations where they were unable to reproduce published results [106]. Similarly, a separate online survey conducted by *Nature* in 2016, which involved 1,576 researchers, found that 70% of respondents had attempted and failed to replicate experiments conducted by other scientists, and more than half had experienced difficulties reproducing their experiments [107, 108]. The presence of irreproducible results escalates project costs, prolongs development timelines, and erodes trust from industry stakeholders and investors. This issue is particularly prominent when the industry

excessively relies on academic institutions that may lack the requisite expertise and infrastructure. Recent findings indicate that approximately US$28 billion is spent annually on irreproducible preclinical research in the United States [109].

Despite successful evaluations *in vitro* and *ex vivo*, it is crucial to exercise caution when interpreting positive outcomes from preclinical data and extrapolating efficacy to human clinical trials. Effectiveness demonstrated in animal studies does not necessarily guarantee efficacy in humans [110–112]. Numerous instances exist where products based on nanomedicine demonstrated promising outcomes but ultimately failed in human trials [113, 114]. There exists a notable disparity between the number of positive preclinical studies and the successful translation of nanomedicine from preclinical to clinical outcomes. This disappointing result renders their commercial translation a risky, expensive, and uncertain endeavor for pharmaceutical companies. Despite significant and urgent unmet clinical needs in areas such as clinical oncology, the risks involved in translating many nanomedicines remain unappealing for a considerable industrial sector. The lack of consistency in translation from bench to bedside can be attributed to several factors: (1) a pervasive skepticism regarding the reliability and reproducibility of academic research; (2) challenges in translating particle dosing established for *in vitro* cell culture studies to *in vivo* tumor models; (3) absence of validation for cancer cell lines, including issues such as contamination, irrelevant culture conditions, and assay parameters; (4) absence of standardized methods and protocols to facilitate valid comparisons across different studies; and (5) significant disparities between outcomes observed in preclinical animal models and results from clinical trials involving humans [88, 114–116]. Many rodent tumors are induced ectopically by injecting humanized tumor cells, resulting in rapid growth and the development of large masses over relatively short periods, which contrasts sharply with the slower, more complex tumor development observed in humans [117–119]. This discrepancy leads to rodent tumors that, if scaled allometrically, would be of a much larger size compared to human tumors and possess substantially different physiological characteristics. The current small rodent models thus fail to accurately replicate human tumor development. Unfortunately, there are currently no large animal tumor models available that could better mimic the scaling, pathology, and physiology observed in human conditions. Therefore, it is essential to establish convincing proof of concept and critically assess efficacy through multiple preclinical models that accurately represent various aspects of the clinical disease, ensuring the reproducibility of findings. Nanomedicine must undergo rigorous and comprehensive testing in preclinical protocols. Careful selection of preclinical *in vivo* models is imperative to ensure they closely mimic human pathological conditions. Bias in the outcomes of preclinical studies should be mitigated through blinding or appropriate randomization strategies, thereby enhancing predictability for clinical translation.

Another factor contributing to the complexity of nanomedicine outcomes is the variability and complexity of disease pathophysiology, which can influence the distribution, accumulation, and retention of nanomedicines. There has been considerably less research focused on thoroughly understanding the relationships between the behavior of nanomedicines and the pathophysiological conditions of diseases. The effectiveness of nanomedicines is also influenced by the pathophysiological conditions of patients. For example, in cancer patients, aspects of tumor vascularization

can vary significantly within a tumor, among tumors in a patient, and even more so among different patients [120]. Therefore, it is crucial to consider the interplay between disease pathophysiology and the heterogeneity of diseases in humans, as well as the physicochemical characteristics of different nanomedicines in overcoming biological barriers, thereby enabling improved drug accumulation and reduced distribution to non-target sites. Additionally, ethical, economic, and genetic differences pose additional challenges to the development of appropriate animal models, which should be addressed by establishing reliable *in vitro* models that accurately mimic the properties and functions of human organ systems. The unpredictability of nanomedicine outcomes in human clinical trials is also due to its unique pharmacokinetic (PK) properties, tissue distribution patterns, accumulation at target sites, and release kinetics. These distinctive characteristics cause nanomedicines to exhibit different behaviors in animals compared to humans. Additionally, nanoparticles are susceptible to the formation of a protein corona due to their size, shape, and surface charge [121]. This phenomenon can sometimes result in accelerated clearance of nanoparticles or their accumulation in the spleen or liver, compromising their target specificity. It is essential to thoroughly investigate the distribution of nanomedicines at both tissue and cellular levels. Understanding the correlation between the properties of the delivery system and its *in vivo* behavior in animals versus humans is crucial for the successful translation of nanomedicines.

Here are some key issues listed below:

1. **Reproducibility in Preclinical Research**
   The research community has a tendency to prioritize positive and noticeable data, sometimes at the expense of rigorous technical outcomes. This bias toward positive results can lead to irreproducible preclinical research. Irreproducible results increase project costs, delay development, and erode trust among industry stakeholders and investors.
2. **Distrust in Academic Research**
   There is a general skepticism surrounding the reliability and reproducibility of academic research, which may deter pharmaceutical companies from investing in the translation of nanomedicines developed in academic settings.
3. **Difficulty in Translating *In Vitro* Results to *In Vivo* Models**
   Determining appropriate particle dosing for *in vivo* tumor models based on *in vitro* cell culture studies poses a challenge. The transition from cell culture to animal models may not accurately reflect the dosing requirements, leading to discrepancies in efficacy.
4. **Issues with Cancer Cell Lines**
   Lack of validation of cancer cell lines, contamination, and irrelevant culture and assay conditions can undermine the reliability of preclinical studies. Inaccurate representation of tumor biology in cell lines may contribute to the failure of translation to human trials.
5. **Lack of Standards and Methods**
   The absence of standardized protocols and methods hampers the validity of inter-study comparisons. The variability in experimental conditions

makes it difficult to assess the reproducibility and reliability of preclinical findings.

6. **Formation of Protein Corona**
   Nanoparticles can interact with proteins in biological fluids, leading to the formation of a protein corona. This corona can influence the pharmacokinetics and biodistribution of nanoparticles, potentially compromise the specificity of nanomedicines for their intended targets. The presence of a protein corona can also affect the clearance of nanoparticles from circulation, leading to faster clearance or deposition in organs such as the spleen or liver.
7. **Discrepancies between Animal Models and Human Studies**
   Ethical and economic considerations, as well as genetic variability, pose challenges in developing appropriate animal models that accurately represent human disease pathophysiology. Preclinical animal models, particularly rodent models, may not accurately replicate human tumor development. Tumors generated in rodents through large injections of humanized tumor cells may differ substantially in size and physiology from human tumors. Existing small rodent models may not adequately represent the complexity of human tumor biology and physiology.
8. **Heterogeneity of Disease**
   Disease pathophysiology can influence the distribution, accumulation, and retention of nanomedicine within the body. Diseases, especially cancer, exhibit significant heterogeneity both within individual tumors and among different patients. Variations in tumor microenvironment, genetic factors, and disease progression can impact the efficacy of nanomedicine in targeting and treating the disease. Variations in factors such as tumor vascularization, tissue permeability, and microenvironmental conditions can influence the biodistribution of nanoparticles and their ability to reach target sites.

### 1.5.1 Strategies to Improve Preclinical Outcomes

1. **Need for Convincing Proof of Concept**
   - Before proceeding to clinical trials, nanomedicines must demonstrate convincing proof of concept and efficacy in multiple preclinical models.
   - It's essential to critically assess efficacy in preclinical studies and ensure the reproducibility of findings.
2. **Selection of Preclinical Models**
   - Preclinical *in vivo* models should closely mimic human pathological and physiological conditions to improve the likelihood of successful translation.
   - Careful consideration is needed when selecting animal models to ensure they represent relevant aspects of the clinical disease.
   - Establishing a correlation between the properties of nanomedicine delivery systems and their behavior in animal models versus humans is crucial for successful translation.

3. **Enhanced Study Design and Rigorous Preclinical Testing**
   - Nanomedicines must undergo rigorous and comprehensive testing taking into account the variability in disease pathophysiology and patient characteristics in preclinical protocols to assess efficacy and safety. This can help identify promising candidates for clinical trials and optimize treatment strategies.
   - Reliable *in vitro* models that mimic human organ systems are needed to complement animal studies and provide insights into nanomedicine performance.
   - In addition to *in vitro* and *ex vivo* evaluations, *in vivo* studies should involve diverse preclinical models to capture various aspects of disease pathology.
   - Ensuring adequate sample sizes and appropriate controls.
   - Conducting blinded and randomized studies to reduce bias.
4. **Quality Control of Biological Reagents and Materials**
   - Using well-characterized and validated reagents.
   - Regularly verifying the quality and consistency of materials.
   - Establishing centralized repositories for critical reagents to ensure their reliability.
5. **Pharmacokinetics and Tissue Distribution**
   - The distinct PK properties of nanomedicine compared to conventional drugs contribute to the unpredictability of their outcomes in human clinical trials.
   - The physicochemical characteristics of nanomedicine, such as particle size, surface charge, and surface functionality, play a crucial role in overcoming biological barriers, improving drug accumulation at target sites while reducing distribution to non-target tissues. Understanding these properties is essential for predicting the efficacy and safety of nanomedicines in humans.
6. **Investigation of Tissue and Cellular Distribution**
   - Thorough investigation of the distribution of nanomedicines at the tissue and cellular levels is necessary to understand their behavior *in vivo.* This includes assessing factors such as particle size, shape, surface charge, and composition, which can influence their interactions with biological systems.
7. **Control of Bias in Preclinical Studies**
   - Bias in preclinical studies can skew results and affect the predictability of clinical translation.
   - Implementing blinding or appropriate randomization strategies can help control bias and improve the reliability of preclinical findings.
8. **Collaborative Efforts and Training**
   - Promoting collaboration between academia and industry to share best practices.
   - Investing in training programs to improve the skills and knowledge of researchers.
   - Encouraging open communication and data sharing to facilitate reproducibility.

9. **Transparent Reporting**
   - Encouraging the publication of negative and null results to provide a complete picture of research outcomes.
   - Implementing guidelines for transparent reporting of methods and results, such as the ARRIVE guidelines for animal research.

The challenges in translating nanomedicine from bench to bedside are multifaceted and encompass various factors contributing to the lack of consistency in successful translation. Translating preclinical efficacy to clinical outcomes in nanomedicine requires a concerted effort to improve the reliability and relevance of preclinical models, to establish standardized methods and protocols, and to enhance collaboration between academia and industry. By focusing on rigorous study design, quality control of materials, collaborative efforts, and transparent reporting, the scientific community can enhance the reliability of preclinical research and increase the likelihood of successful clinical translation. It's essential to critically evaluate preclinical data, ensure convincing proof of concept, rigorously test nanomedicines in relevant preclinical models, and control bias in study design and execution. Comprehensive understanding about complex interplay between disease pathophysiology and nanomedicine performance is essential for advancing the clinical translation of these promising therapeutic agents. Developing more clinically relevant animal tumor models that better replicate human tumor biology and physiology is crucial for improving the translational success of nanomedicine in oncology and other therapeutic areas. Implementing these strategies will not only reduce costs and delays but also build trust among stakeholders and improve the predictability and success rate of clinical translation in nanomedicine.

## 1.6 NANOTOXICOLOGY

The distinctive characteristics of nanomedicines, such as their small particle size and surface reactivity, represent a dual-edged feature that significantly influences their interactions within living organisms. The complexity inherent in nanoparticles renders them somewhat unpredictable in terms of potential toxicity profiles. With the rapid advancement of nanomedicine, there is a growing focus on assessing biocompatibility and nanotoxicity [122, 123]. In this context, it becomes crucial to comprehend the properties of nanomedicines, their interactions with biological systems, and the associated hazards and risks. As previously discussed, upon administration to patients, nanomedicines exhibit unique biodistribution properties due to their ultra-small particle size and surface functionality. Consequently, they demonstrate notably higher uptake in various organs, leading to drug overexposure in these organs and resulting in undesirable side effects. Nanoparticles possess the capability to traverse biological barriers, including cellular membranes, as well as barriers such as the blood–brain barriers *in vivo* [124–126]. This presents significant opportunities to access localized regions that conventional materials cannot reach. However, there are potential risks associated with uncontrolled migration and deposition of nanoparticles within tissues, which could adversely affect patients. For instance, certain nanoparticles have a tendency to accumulate in lymphoid organs, while polymeric nanoparticles may accumulate in the kidneys. Additionally, the clearance of

nanoparticles is influenced by their size; smaller nanoparticles, below 20–30 nm, are rapidly cleared through renal excretion, whereas particles larger than 200 nm are more efficiently taken up by the mononuclear phagocytic system, located in organs such as the liver, spleen, and bone marrow [127–130]. Furthermore, the use of novel excipients in the development of nanomedicine, with unknown safety profiles, may pose challenges to its further advancement. Various species exhibit different levels of tolerance to the same nanomaterials, potentially leading to serious adverse effects in certain species. Early preclinical PK and biodistribution studies, along with histopathological studies, are advisable to assess organ drug exposure and toxicity. Additionally, studies are needed to investigate how nanomaterials penetrate cells and tissues, as well as their respective biodistribution, degradation, and excretion patterns. The immunological response presents an additional hurdle in nanomedicine development. While hypersensitivity reactions occur in a small proportion of individuals following its administration, they can be severe and occasionally life-threatening [131–133]. Consequently, immune toxicity must be assessed, particularly in immune-sensitive species like rabbits. Additionally, *in vitro* complement binding and cell interaction assays can provide valuable insights. Consequently, a new field of toxicology, known as nanotoxicology, has emerged to study the effects of nanomaterials resulting from their interaction with biological systems. A comprehensive understanding of nanotoxicology is essential for the safe and effective development of nanomedicines, ensuring their translational success from bench to bedside.

## 1.7 CLINICAL DEVELOPMENT FEASIBILITY

Most of the nanomedicine research are focused on cancer therapies and despite the large number of publications, the translation of these studies into clinical applications has been disappointing. Many times, we see that opening paragraph of research publications talks about exploiting an EPR effect as a reason for using nanomedicine for cancer therapy. One of the reasons for this disappointment is the over reliance on the EPR effect [114, 134–136]. This concept hinges on the leakiness of tissues and blood vessels, characterized by tortuous, aberrant, and permeable pores that facilitate the passive diffusion of certain nano-sized structures. Numerous papers have been published based on this premise, and several clinical trials have been conducted using various types of nanoparticles. While the EPR effect theoretically allows for passive accumulation of nanoparticles in tumor tissues, a meta-analysis paper published in 2016 revealed that the actual delivery of nanocarriers to tumor tissues has been found to be minimal, with rates close to 0.7% [137, 138]. The EPR concept has faced notable criticism in recent years. Despite assertions of successful preclinical outcomes, the practical efficacy of the EPR effect in translating preclinical success into promising clinical results in humans remains unclear.

One of the primary challenges with the EPR effect is the heterogeneity of tumors, both within and between patients, which affects the occurrence and extent of the effect. Tumors evolve over time, leading to changes in their pathophysiological patterns that can significantly influence the EPR effect. For instance, prior study conducted by Radicchi et al., EPR effect monitored in a cohort of tumor-bearing mice over four consecutive weeks. Throughout this period, various pathophysiological

patterns within the tumors changed, significantly impacting the manifestation of the EPR effect [139, 140]. Moreover, properties of the drug carriers, such as particle size, surface charge, shape, and stability, also play a pivotal role in the performance of EPR-based delivery systems and must be meticulously designed and validated. Therefore, adopting a one-size-fits-all approach to designing nanomedicines is unlikely to yield clinically beneficial outcomes. The exploitation of the EPR effect extends beyond cancer diseases, and it's important to recognize that EPR-based nanomedicine distribution may not be applicable to all disease conditions. It's improbable that the EPR effect will be present in all disease contexts. Furthermore, EPR is not the sole determinant of nanomedicine efficacy; other mechanisms, such as active transport processes, influence cellular uptake and drug release within target tissues. Studies have shown that despite the presence of continuous tumor vasculature, most nanoparticles are absorbed by tumor tissues, confirming the existence of active transport mechanisms [140, 141]. In summary, while the EPR effect has been a significant focus in nanomedicine development, its translation to clinical applications faces challenges due to tumor heterogeneity, variability in drug carrier properties, and the presence of alternative mechanisms for nanoparticle uptake. Moving forward, researchers must consider these factors and explore alternative strategies to optimize the clinical translation of nanomedicines.

Most of the nanomedicine developed till date are intended for its non-oral administration and circulating half-life for most of them is less than 24 hours. The short circulating half-life of most nanomedicines following intravenous administration presents a significant challenge, as rapid clearance by liver Kupffer cells and spleen macrophages limits their accessibility to disease sites. Extending the circulation time of nanomedicines has been a major focus of research, and the most successful approach thus far involves the use of hydrophilic polymers, particularly polyethylene glycol (PEG), grafted onto the nanoparticle surface [129, 142–144]. This polymeric stabilization minimizes serum protein opsonization and corona formation, reducing particle-cell and particle-particle aggregations, and inhibiting scavenging uptake by the mononuclear phagocyte system (MPS). Enhancing circulation times and improving accessibility, whether passively or actively targeting disease sites, is crucial to enhancing the dosing of nanomedicines to target sites. There has been a noted inconsistency regarding ligand-targeted nanomedicine, with minimal advancement achieved thus far. Several factors contribute to this discrepancy, including target expression, specificity, accessibility, pathophysiological barriers, and the optimal functionalization of ligands on nanomedicine [145–147]. Thorough characterization and studies on the distribution and accumulation of nanomedicine at the tissue and cellular levels are crucial factors to consider for clinical validation of targeted therapy. In summary, addressing the challenge of short circulating half-life and improving targeting efficiency are critical for enhancing the clinical translation and efficacy of nanomedicines. This requires further research into novel strategies for extending circulation times and optimizing ligand-targeted approaches, as well as comprehensive characterization studies to validate nanomedicine distribution and accumulation *in vivo*.

Addressing the challenge of conducting larger and expensive clinical trials is crucial for the successful development and adoption of nanomedicines. To ensure

alignment with clinical challenges, it is imperative to establish clear strategic directions regarding clinical development pathways and the resulting benefits for patients. Firstly, the selection of endpoints in clinical trial design must align with anticipated patient benefits that government bodies and payors are willing to support. Clinically relevant outcomes, such as disease cure, enhancement of patient lifestyle, or delay in disease progression, are desirable but entail significant time, investment, resources, and a large patient population. These factors introduce uncertainty, often influencing the approaches taken by industry and investors. It is crucial to assess these points upfront before commencing clinical trials, as they largely hinge on expected variations in trial endpoints. Having an appropriate preclinical platform available may aid in predicting clinical outcomes in patients. Variability in trial outcomes can also be addressed through the implementation of biomarkers predictive of therapeutic response, which should be identified early in the preclinical development stage. Surprisingly, biomarkers are not being utilized to tailor nanomedicine treatments for patients. While nanomedicines like doxorubicin liposome and Abraxane have demonstrated significant therapeutic advantages and enhanced overall quality of life, this has come at the expense of mitigating their toxicity profiles. However, the therapeutic potential of these drugs has not yet been optimally assessed. Integrating biomarkers into clinical trials can enhance patient stratification, improve trial outcomes, and facilitate personalized medicine approaches.

## 1.8 CURRENT REGULATORY CHALLENGES

Nanomedicines have significant potential to increase the growth of the pharmaceutical market and improve the health benefits. However, the existing scientific and regulatory gap for nanomedicines is substantial and complex. Nanomedicines encompass various types of nanomaterials and nanostructures, such as polymeric nanoparticles, which include numerous novel polymeric materials. It is crucial to note that the biocompatibility and safety of these materials are highly contingent upon their properties, including molecular weight, viscosity, conjugation pattern, polydispersity, and molecular structure [148]. This complexity further complicates the establishment of suitable regulatory protocols and standards. Moreover, there is a scarcity of specialized manufacturing units for nanomedicines like liposomes and polymeric nanoparticles, and products manufactured in these units may seek marketing approval in multiple countries. Regulatory authorities should collaborate to develop uniform standards and testing protocols to facilitate faster approval of nanomedicines across countries. These efforts are essential to realizing the full potential of nanomedicines in improving healthcare outcomes.

Presently, all nanomedicines undergo regulation and approval processes based on the traditional frameworks established by the primary regulatory authorities in each country (such as the USFDA, HC, TGA, and EMA) [99, 116, 149]. However, this approach poses challenges to the advancement of nanomedicines and slows down the approval timelines for these products. Given the intricate nano-sized structures, multifunctionality, and unknown interactions of nanomedicines with cells and tissues, conventional standards are not suitable for verifying the quality, safety, and efficacy of nanomedicines for clinical applications. To address these challenges,

validated protocols and standards tailored to the complexity, route of administration, distribution, and safety profile of nanomedicines are needed. Additionally, the commercialization of nanomedicines is indeed influenced by government policies, which can vary significantly from one country to another. Global government policies and regulations for nanomedicines are underdeveloped, leading to inconsistencies in regulatory frameworks from country to country. This diversity in regulatory frameworks can hinder the potential of nanomedicines by complicating the approval and regularization process. The lack of established or developed worldwide government policies, ethics, and regulations further exacerbates this challenge. This inconsistency again hinders the potential of nanomedicines and complicates approval processes. Another issue contributing to the complexity of nanomedicine regulation is the confusion, disagreement, and ambiguity surrounding terminology related to nanotechnology. Terms like "nanotechnology," "nanoscience," "nanodrug," "nanomaterials," "nanomedicines," and "nanoscale" are often used interchangeably or inconsistently [74, 99, 149]. The indiscriminate use of the "nano" prefix for various purposes such as securing research funding, patent approvals, fundraising, political campaigns, or publication purposes further exacerbates these challenges. This practice can create confusion and inconsistencies in the understanding and application of nanotechnology-related concepts and principles among scientists, policymakers, regulators, patent officers, and legal experts. Establishing nomenclature, technical specifications, standards, guidelines, and best practices is crucial to advancing nanotechnology safely and responsibly. Establishing consensus on terminology and regulatory frameworks will help streamline the development, approval, and commercialization of nanomedicines while ensuring their safety and efficacy. However, there must be a fine balance between ensuring quality and avoiding overregulation, which can increase development costs and shorten the lifespan of patents, hindering innovation.

The emergence of lower-cost generic versions of nanomedicines as patents expire adds complexity to regulatory approvals. While generic manufacturers aim to replicate innovator products using identical processes and technologies, achieving an exact replica is challenging due to the complexity of nanomedicine technology. Variations in manufacturing processes and formulations can result in generic products with different physicochemical properties such as size, size distribution, surface properties, drug loading, release profile, aggregation status, and stability [150–152]. These differences could significantly impact patient safety and efficacy. Conventional standards may not adequately evaluate these differences, posing challenges for regulators, national competent authorities, and formulary administrators in determining interchangeability/substitutability between originator and generic drugs. For instance, there have been reported instances where a generic version of a nanomedicine was found to be pharmaceutically equivalent to the innovator product *in vitro* but failed to demonstrate equivalency *in vivo* [84, 153, 154]. Identifying the reason for such failures using conventional standards and protocols is challenging. While conventional standards and risk-benefit evaluations may suffice for approving innovator drug products, they may not adequately evaluate and differentiate the quality differences in generic versions against innovator products, thus affecting the safety and efficacy of the generic product. Therefore, addressing these challenges

requires tailored regulatory approaches and standards specific to nanomedicines to ensure patient safety and efficacy while facilitating the approval of generic versions.

The FDA has indeed taken significant steps to enhance understanding and regulate the application of nanotechnology across various industries. In the realm of nanomedicines specifically, the FDA has made notable progress by publishing product-specific guidance documents for certain nanomedicine products. For instance, the FDA has issued guidance documents for nanomedicine products such as liposomal formulations of doxorubicin hydrochloride [155], irinotecan hydrochloride [156], sodium ferric gluconate colloidal complex [157], and iron sucrose [158] over the past decade. These guidance documents provide important regulatory information and recommendations for manufacturers seeking approval for these specific nanomedicine products. Additionally, a draft guidance published in 2018 specifically addresses general liposomal products, focusing on technical aspects of manufacturing liposome drug products [159]. However, it does not provide recommendations on non-clinical and clinical studies, indicating the need for further guidance in these areas to facilitate the development and approval of liposomal nanomedicines. Moreover, the FDA has also developed regulatory guidelines for the use of nanomaterials in other sectors such as foodstuffs [160], cosmetics [161], animal feed [162], and drug products [163]. These guidelines serve as crucial resources for stakeholders in these industries, offering insights into regulatory considerations and requirements related to the use of nanotechnology.

The regulatory landscape for nanomedicines varies across different countries, with some countries having specific guidelines in place while others are in the process of developing them. The FDA's guidance published in 2017 (updated in 2022) for the industry regarding generic nanomedicines represents a significant step toward addressing the regulatory considerations and pathways for these complex products [163]. This guidance discusses scientific viewpoints, potential regulatory pathways such as the 505(b)(2) or abbreviated 505(j) pathway, and bioequivalence (BE) considerations for generic nanomedicine applicants. From a quality perspective, the guidance outlines 11 risk factors to consider as part of the product's CQAs and assessment of the nanomaterial, including adequacy of characterization of the material structure and its function, complexity of the structure, effect of particle size on PK parameters, understanding the *in vivo* release mechanism based on the material physicochemical properties, predictability of *in vivo* release, physical and chemical stability, manufacturing and analytical methods, physical state of the material upon administration, route of administration, potential impact of manufacturing changes and dissolution, bioavailability, distribution, biodegradation, accumulation, and their predictability based on physicochemical parameters and animal studies. However, it also acknowledges that additional quality attributes may also apply to nanomaterials in drug products, depending on the particular drug product (e.g., route of administration), its indication, and patient population due to the diversity of nanomaterial formulations and drug-release mechanisms. Regarding the filing pathway, the guidance suggests that evidence of comparable PK parameters in blood/plasma from conventional BE studies alone may not be sufficient to establish BE between the generic and the reference listed drug (RLD), depending on the route of administration and nanomaterials employed. A report published in 2019 raised

concerns about the suitability of the 505(j) pathway for nanomaterial-containing drug products, citing challenges in demonstrating pharmaceutical equivalence (PE challenges), adequately characterizing these products to support a determination of sameness, differences in manufacturing processes leading to product variation, differences in PK and pharmacodynamic properties due to varying physicochemical characteristics and BE assessment is often more challenging than for conventional formulations [164]. This indicates the need for a more comprehensive approach to demonstrating BE for generic nanomedicines. Overall, while the FDA's guidance represents progress in addressing regulatory considerations for generic nanomedicines, ongoing efforts and collaboration between regulators, industry stakeholders, and researchers are needed to address the unique challenges associated with these complex products and ensure patient safety and efficacy.

Similarly, the European Medicines Agency (EMA) has been proactive in recognizing and addressing the unique challenges posed by nanomedicines. Since 2005, various initiatives and platforms have been established to facilitate discussions and address regulatory challenges in the field of nanomedicine. One notable initiative is the European Technology Platform on Nanomedicine (ETPN), established in 2005 by the European Commission and industry stakeholders [165]. The ETPN aims to support the successful translation of nanomedicines by shaping and supporting public funding in promising areas of nanomedicine research and designing technical infrastructure such as the Nanomedicine Translation Hub. In 2006, EMA published a reflection paper advising applicants to contact the agency in the early stages of development in the absence of specific guidance [166]. This reflected EMA's recognition of the need for early engagement with developers of nanomedicines to address regulatory considerations effectively. Subsequently, in 2009, EMA established an Ad-Hoc Nanomedicines Expert Group consisting of experts from disciplines such as quality, safety, and kinetics [167]. The objective of this group was to formulate guidelines specific to nanomedicines. Later, this group expanded into an International Regulators Subgroup on Nanomedicine, a joint initiative launched by regulators from Canada, Europe, Japan, and the United States. In 2011, EMA established a nanomedicine drafting group tasked with developing a series of reflection papers on specific aspects of nanomedicines, including micelles, liposomes, and nanoparticles [168–171]. These reflection papers serve to provide guidance and recommendations but are not legally binding across the European Union (EU). Currently, the EU regulates nanomedicines using risk/benefit-analysis principles, emphasizing the importance of evaluating the risks and benefits associated with these products to ensure their safe and effective use. Despite the progress made, ongoing efforts are needed to further develop regulatory frameworks and guidelines to address the unique challenges posed by nanomedicines and promote their successful translation and commercialization in the EU market.

In the United Kingdom, the Medicines and Healthcare products Regulatory Agency (MHRA) manages the approval of nanomedicines on a case-by-case basis. Researchers are encouraged to communicate with the MHRA for support throughout the development process, although specific guidelines for nanotechnology-based products are lacking. Similarly, Health Canada supports and advises manufacturers during the development process of nanomedicines by assessing their risks and

properties. In India, efforts are underway to establish regulatory guidelines for nanomedicines. The government and the Department of Science and Technology have formed a working group responsible for regulating and drafting guidelines for nanotechnology-based products. In 2019, the Indian government published the first guidelines for nanomedicine regulation, covering the development of new drugs and their comparison with existing entities [172]. In Japan, the Ministry of Health, Labor and Welfare (MHLW) and the Pharmaceuticals and Medical Devices Agency (PMDA) are responsible for regulating nanomedicines. Specific guidelines for the regulation of liposome-based drug products were published in 2016 [173]. Nanomedicines are legislated on a case-by-case basis under the Pharmaceutical Affairs Law framework, often in close collaboration with international regulatory bodies such as the EMA.

Overall, the regulatory environment for nanomedicines is evolving globally, with various countries taking steps to establish guidelines and frameworks to ensure the safety and efficacy of these innovative products. Nevertheless, Global regulatory standards for nanomedicines should be established through collaboration among key countries with vested interests in the field. While draft guidances and papers published by regulatory agencies represent progress, they are often not sufficient to address the complexities and challenges unique to nanomedicine development. Standardizing protocols and requirements across the various stages of nanomedicine development is essential to encourage industry investment and participation in the field. Achieving consensus on procedures, assays, and protocols for the development of nanomedicines is crucial for advancing the field and ensuring the safety and efficacy of these innovative products. However, this task is complicated by the lack of maturity, technical expertise, and central coordination within regulatory bodies. It requires coordinated efforts from multiple stakeholders, including regulatory agencies, industry partners, academic researchers, and international organizations. No single entity can address this challenge alone, emphasizing the importance of collaboration and cooperation in advancing the regulatory framework for nanomedicines. This collaborative approach would help harmonize regulatory requirements and streamline the development and approval processes for nanomedicines.

## 1.9 CONCLUSION

Overall, this chapter captures the essence of the rapidly evolving field of nanomedicine and its promising potential in clinical applications, particularly in cancer treatment. The examples of liposomal-based drug delivery and mRNA-based therapeutics serve as inspiring benchmarks, illustrating the importance of patience and perseverance in the development of new technologies. The timeline of liposomal drug delivery, from its proposal in the early 1980s to the recent approvals of Vyxeos and Onivyde, underscores the lengthy and iterative process involved in bringing these innovations to market. Similarly, the journey from the initial proof of concept for mRNA-based therapeutics in the 1990s to the recent approvals of COVID-19 vaccines highlights the transformative impact of continuous research and development efforts. This achievement also reminding us of the importance of persistence and strategic focus in advancing these innovations toward meaningful clinical outcomes.

Emphasis on the need to maintain focus on translating research efforts into clinical practice is crucial. As the nanomedicine market expands, it is essential to explore novel approaches and technologies while remaining mindful of the complexities and challenges involved in clinical translation. Robust infrastructure, guidelines, and protocols for the manufacturing and characterization of nanomedicine are required. Understanding the early stages of nanomedicine preparation and assessing the feasibility of industrial-scale production, ensures both technical and financial aspects in the development process. This approach makes sure that resources are allocated efficiently and that realistic expectations are set for the development and scaling up of nanomedicine production. Diverse array of tools available for characterizing the chemical properties of nanomedicines and quality control and meticulous characterization in the development and clinical application of nanomedicine is critically important. These techniques play a crucial role in ensuring the reproducibility and consistency of nanomedicine formulations, as even minor changes in properties can have significant implications for drug distribution and bioavailability. Despite promising preclinical results, the clinical translation remains slow, biased, and often unsuccessful. Therefore, it is crucial to take informed decisions not only from a CMC perspective but also in terms of preclinical experimental setup and clinical trial design to maximize the potential benefits for patients. Rigorous evaluation at every stage of development with innovative preclinical protocol and testing models, such as organs-on-chips offers more accurate assessments of the safety and efficacy of nanomedicine formulations. It is important to have a rational roadmap and strategic planning for the clinical translation of nanomedicine to maintain credibility within the scientific community and among stakeholders. This includes addressing challenges in technology translation and considering investment risks and returns early in the development process. We must move away from continuously making increasingly complex nanomedicine and we must focus on smart strategies to translate nanomedicine into a clinically applicable therapeutic. This requires rational and realistic thinking, effective regulatory policies, and collaborative efforts from consortia comprising academics, biologists, clinicians, pharmaceutical companies, policymakers, and regulatory authorities. No single entity, organization, or institution can address these challenges in isolation.

The trajectory of nanomedicine remains uncertain, whether it will sustain its current hype and evolve into a thriving industry or continue making gradual contributions to healthcare. The outcome is still unfolding, akin to other emerging technologies. It's challenging to distinguish hope from hype, but it's crucial to temper exaggerated claims and overstated experimental outcomes to ensure the longevity of the field. Blind optimism without scientific credibility undermines future advancements. By working together, we can accelerate progress and shape the future.

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# 2 Regulatory Guidance on CMC of Nanoparticles

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## 2.1 INTRODUCTION

### 2.1.1 Definition and Importance of Nanoparticles in Pharmaceuticals

As such, there is no guidance or regulatory definition of the word nano. Nanoparticles are mostly inorganic, organic, or polymeric particles with at least one size dimension in the nano-scale range (1–1000 nm) [1].

The active ingredient is either dissolved, dispersed, entrapped, encapsulated, or attached to a nanoparticle matrix [2]. Nanoparticles can be engineered to have different properties such as size, shape, charge, and surface chemistry. Nanoparticles are designed to reduce toxicity and improve efficacy in broader terms [3]. This can be achieved using passive and active drug targeting, ligand-based targeting or use of magnetism. Although intravenous, intramuscular and subcutaneous routes are choice for administering nanoparticles, various other routes of administrations have also been explored such as oral, nasal, transdermal, ocular, inhalation, etc. [4].

### 2.1.2 Overview of Chemistry, Manufacturing, and Control (CMC) in Drug Development

The primary objective of CMC is to assure the quality of the finished product during all phases of development. It is mandatory for all innovators as well as generic companies to establish tests and procedures to ensure quality of the product during in-process, at finished product stage and during stability to meet the regulatory agency standards and expectations [5].

CMC involves defining protocols and procedures to be followed for smooth manufacturing of the products and define control in terms of specifications to ensure product quality and safety. CMC starts right from stage of drug discovery and continues during entire drug product development life cycle [5].

The more robust the protocols and standard operating procedures, less will be the deviations during the manufacturing process. Nanoparticles differ from conventional formulations in terms of extensive characterization requirements that are costly and time consuming. Also, they suffer from lack of skilled resources and variability in the analysis. Hence, identification of *in vitro* tools that can be adopted both for quality control purpose and can also be used in identifying the product failure is need of time [5, 6].

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### 2.1.3 Relevance of Regulatory Guidance in Ensuring the Safety, Efficacy, and Quality of Nanoparticle-Based Drugs

Like all other drug delivery platforms, regulation is prerequisite in the nanotechnology-based drug products as well. Regulatory guidance on the Chemistry, Manufacturing, and Controls (CMC) of nanoparticles is crucial for ensuring the safety, efficacy, and quality of product after every batch. Nanoparticles exhibit distinct properties and behaviors at the nanoscale. There is still a limited understanding of how nanoparticles behave in biological systems and the environment. This makes it difficult to predict their long-term effects and potential risks. The lack of standardized nomenclature, test methods, and characterization techniques for nanoparticles complicates regulatory processes. Consistent and universally accepted standards are essential for accurate assessment and comparison. Nanoparticles often exhibit increased biological activity and unique properties compared to their larger counterparts. These differences can lead to novel risk profiles that are not well understood, posing challenges for safety assessments [7]. Existing regulatory frameworks may not be fully equipped to address the specificities of nanomaterials. Adapting these frameworks or developing new ones to effectively regulate nanoparticles is a complex task. Effective regulation of nanoparticles requires expertise from multiple disciplines, including chemistry, biology, toxicology, and materials science [8]. The shortage of trained personnel with this interdisciplinary knowledge can hinder regulatory efforts. The unique properties of nanoparticles raise ethical and legal questions, particularly in clinical trials and patient use. Ensuring informed consent and addressing potential public health implications are critical but challenging aspects [9].

These challenges highlight the need for ongoing research, collaboration, and adaptation of regulatory practices to ensure the safe and effective use of nanotechnology.

## 2.2 NANOPARTICLES IN DRUG DEVELOPMENT

### 2.2.1 Types of Nanoparticles

#### 2.2.1.1 Liposomes

Liposomes are single- or bi-layered lipidic particles in which drug is either entrapped or embedded. The lipids can be synthetic or natural made of phospholipids, cholesterol, and phosphatidyl choline. Liposomes being in the nanosized range need to be characterized by particle size, surface charge, and enthalpy [10]. Liposomes are mainly used in the diseases such as cancer where targeting is required to reduce toxicity and enhance efficacy. Different surface modifications with hydrophilic carriers such as polyethylene glycol (e.g., Doxil) can lead to stealth behavior of liposomes enabling longer circulation times in the blood [11].

#### 2.2.1.2 Solid Lipid Nanoparticles (SLNs)

SLNs are the combination of liposomes and polymeric nanoparticles. SLNs have particle size below 1 micron and consist of a solid lipid matrix, where the drug is embedded [12]. They were prepared as alternative to conventional emulsions as

colloidal drug carriers. They provide controlled drug delivery and mainly explore in the pulmonary route of administration [12].

#### 2.2.1.3 Polymeric Nanoparticles (PNPs)

PNPs are made from use of biodegradable polymers such as poly lactic-co-glycolic acid (PLGA), polylactic acid (PLA), polycaprolactone (PCL) to name a few. PNPs offer greater stability and high drug loading capacity. They are usually designed to achieve long duration of action via administration through intramuscular or subcutaneous routes particularly in the case of schizophrenia, prostate cancer, and psychotic disorders [13].

#### 2.2.1.4 Dendrimers

Dendrimers are extensively branched large molecules where drug can be entrapped through complexation. The dendrimers are prepared using polymerization reactions and offer large surface for drug loading [14]. They differ from other nanoparticles in terms of size and complexity. Though, limited approved products are available using dendrimers, still it is emerging field in the field of nanotechnology [15].

#### 2.2.1.5 Nanotubes

Nanotubes are formed stepwise due to intrinsic property of peptide molecule where alpha, beta, gamma, and random coils of structure are present. This formation is concentration dependent and reversible. They can be single- or multi-walled depending on the molecular weight [16]. The physical, structural, and thermal characterization of nanotubes is of paramount importance in order to have similar therapeutic properties. USFDA has approved Somatuline (Lanreotide) autogel to be administered subcutaneously and provide effect for up to one month in acromegaly [17].

#### 2.2.1.6 Inorganic Nanoparticles

Iron oxide nanoparticles are class of nanoparticles from inorganic category. Total five iron carbohydrate complex products have been approved by USFDA which differ in their carbohydrate counterpart, physicochemical, and PK properties [17]. Generic companies need to perform extensive characterization as part of CMC along with performing bioequivalence studies to show sameness to innovator product. However, these techniques suffer from variabilities and thus regulatory considerations need to be revisited in order to have a middle-out approach.

Magnetic nanoparticles consist of iron, nickel, and cobalt like metals which can be manipulated by magnetic field and a chemical component that has functionality. They range from nano size to micron size depending on the intended use [18].

#### 2.2.1.7 Polymeric Micelles

Polymeric micelles are self-assembling colloidal dispersions with a hydrophobic core and hydrophilic shell in the size range of 10–100 nm. Nano micelles used to solubilize water-insoluble drugs resulting in a clear aqueous solution [19]. They serve as useful carriers as they minimize degradation of drug, improve permeation of drug through ocular surfaces with minimal or no irritation, leading to enhanced ocular bioavailability and ultimately reduce adverse effects. The nano micelles have

popularly been used in ocular drug delivery systems where suspension products suffer from patient compliance issues [20].

### 2.2.2 Unique Challenges Posed by Nanomaterials in Manufacturing and Control

USFDA identifies medicines that generally have complex active ingredients, formulations, dosage forms, routes of administration, or are complex drug-device combination products as complex products. Additional complexity includes scale-up challenges, unclear regulatory pathways and abnormal challenges in manufacturing or administration processes. Nanoparticles certainly belong to this class, where complexity lies in terms of formulation and manufacturing processes.

#### 2.2.2.1 Formulation Challenges

Formulation of nanoparticles for generic companies suffers from challenges such as matching molecular weight, particle size distribution, surface characteristics like surface charge, membrane properties, drug loading, and drug release kinetics [21].

Nanoparticles are cleared by macrophages which are part of reticuloendothelial systems (RES) and through renal clearance. This phenomenon is dependent mainly on molecular weight, particle size distribution, and surface charge [22].

Matching the molecular weight in the case of generic applicants is critical to ensure similarity in terms of chemistry. However, there lie challenges in terms of synthesis processes which are generally protected by patents and not revealed in the public domain. To have similar chemical properties using alternative route of synthesis is always challenging and require control on the synthesis process.

Particle size similarity is governed by similar size distribution and this can be achieved by population bioequivalence (PBE) studies specified by USFDA in the product-specific guidance's of each product [23]. The $F_2 > 50$ is generally accepted as proof of similarity [24]. However, in the case of complex nanoparticles $F_2$ close to 100 is always preferable as the area under the distribution curve can directly be linked to *in vivo* performance of the two formulations in the case of nanosuspension products [25].

Surface characteristics such as charge and hydrophobicity are critical in nanoparticle stability and the phagocytosis process [26]. Once in circulation, colloidal nanoparticles are coated with various blood proteins to aid in phagocytosis and clearance from the blood. The more hydrophilic surfaces mean less phagocytosis and vice versa [27]. Measurement of surface charge (zeta potential) of the product before and after dilution has been recommended to ensure product similarity for generic formulations.

The membrane properties of the liposomes affect drug release kinetics due to presence of different lipids, polymers, surfactants, and their arrangements in the matrix [28]. The membrane uniformity and rigidity can be assessed by determining phase transition temperature (Tm) and enthalpy ($\Delta H$) of lipid layers [25]. Establishing similarity in both of these parameters ensure similar lipid layer structure and thereby drug release from the liposomes, which is key for generic drug development [25].

Some of the biggest challenges generic companies face while developing nanoparticle formulations is the variability arising from API and excipients. The synthesis route of API can be very different for innovator and generic companies which can

lead to variations in impurity profiles, conformational stability, solubility, and three-dimensional structure in case peptide formulations. Sometimes, polymers used by innovator companies are custom made or proprietary polymers, which then pose different challenge for generic competitors due to patentability issues. Excipients provided by manufacturers and suppliers can be different due to differences in synthesis route. These small differences can significantly impact the formulation development of nanoparticles.

Nanoparticles such as metal-carbohydrate complexes pose two-way challenges for generic applicants in terms of matching the both physiochemical characteristics and PK equivalency. The macrophage uptake of these formulations largely depends on the magnetic properties of the core, interactions of core with carbohydrate ligand and affinity towards plasma proteins. The regulatory requirements of showing bioequivalence in total, bound and unbound content indirectly suggest that generic product should be similar with reference product at each and every time point of PK profile. Finding the biorelevant *in vitro* characteristics to establish *in vitro in vivo* correlation is challenging for these products due to limited understanding of the complex processes involved during the macrophage uptake.

#### 2.2.2.2 Process Challenges

The successful scale-up and manufacturing of nanoparticles is challenging due to complexity in the manufacturing processes involved. The manufacturing of nanoparticles differs from conventional product manufacturing in terms of involvement of critical steps that are not fully scalable from lab scale to pilot scale due to limitations of equipment specifications [29]. Examples include stirring or homogenization speeds, drying times, curing times, lyophilization cycles, and filtration processes.

The manufacturing of nanoparticle mainly involves "top–down" and "bottom–up" approaches. Top–down approaches involve milling processes where coarse particle is converted into nanometer scale. While, bottom–up approaches involve polymerization of monomers into polymers or molecular self-assembly into desired conformations [30].

Many a times product which was successful at lab scale fails at pilot scale due to limited understanding of the above-mentioned phenomenon's happening at the large scale. Optimizing polymer and surfactant concentrations, solvent ratio, and controlling stirring or sonication processes help to achieve desired nanoparticle characteristics.

Nanoparticle process should be as robust as possible to ensure smooth reproducibility and scale-up at commercial scale. For example, in the case of nanoparticles involving lyophilization process, care should be taken to reduce the number of cycles and identification of end points during lyophilization process. This will help to shorten the cycle duration at large scale and improve predictability. Maintenance of consistent temperatures, adequate changes in chamber pressure distribution, addressing heat transfer rate differences, controlling nucleation process variability at different scale and adjustment of freezing and drying parameters are some critical checks required for successful scale up of nanoparticles and reproducing desired characteristics similar to lab scale.

In the case of nanoparticles involving emulsification phases, the manufacturing process parameters should be identified that critically affect the stage wise

performance of product such as phase concentrations, stirring speeds, drying temperatures, solvent quantity, and evaporation rates along with curing process that need special considerations in the scaling up process to ensure consistent product performance [31].

In the case of liposomes, improper scaling can result in change in lamellar structure and particle size distributions. To avoid this, suitable analytical techniques need to be part of quality control at each stage of manufacturing [32]. Slight change in manufacturing steps can result in small or large unilamellar liposomes or multilamellar liposomes. Liposomes are unstable due to high-surface free energy and the tendency to aggregate, which can be mitigated by adding amphipathic molecule such as polyethylene glycol. Microfluidics and continuous flow manufacturing techniques can help achieve uniform liposome size. Drug encapsulation efficiency can be improved by optimizing lipid composition and using active loading methods. Addition of stabilizers and optimizing lyophilization techniques enhances shelf life of liposomes.

Advanced analytical characterization techniques can also be part of the quality control at least at the initial stages of scale-up development to ensure successful scalability.

## 2.3 KEY REGULATORY FRAMEWORKS

The available guidance documents for USFDA, European Union, and regional frameworks are presented in Table 2.1 [33,34]. Also, comparative recommendations for critical parameters are presented in Table 2.2 [35–37].

**TABLE 2.1**
**Regulatory Guidance's for Nanomedicines [34,35]**

| Sr. No. | Name of Guidance | Issue Date | Regulatory Organization | Topic | Status |
|---|---|---|---|---|---|
| 1 | Drug Products, Including Biological Products, that Contain Nanomaterials – Guidance for Industry | 21/04/2022 | Center for Drug Evaluation and Research **(US)** | Chemistry, Manufacturing, and Controls (CMC), Pharmaceutical Quality | Final |
| 2 | Guidelines for Evaluation of Nanopharmaceuticals in India | 03/07/2019 | Central Drugs Standard Control Organization **(India)** | Not specified | Draft |
| 3 | Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation | 05/04/2018 | Center for Drug Evaluation and Research **(US)** | Chemistry, Manufacturing, and Controls (CMC), Pharmaceutical Quality | Final |

*(Continued)*

**TABLE 2.1** ***(Continued)***
**Regulatory Guidance's for Nanomedicines [34,35]**

| Sr. No. | Name of Guidance | Issue Date | Regulatory Organization | Topic | Status |
|---|---|---|---|---|---|
| 4 | Use of Nanomaterials in Food for Animals | 05/08/2015 | Center for Veterinary Medicine **(US)** | Nanotechnology, Animal Food Additives | Final |
| 5 | Data requirements for Intravenous Iron-Based Nano-Colloidal products Developed with Reference to an Innovator medicinal Product | 27/03/2015 | European Medicine Agency **(EU)** | EMA/CHMP/ SWP/620008/2012 | Final |
| 6 | Guidance for Industry: Safety of Nanomaterials in Cosmetic Products | 24/06/2014 | Center for Food Safety and Applied Nutrition, Office of Cosmetics and Colors **(US)** | Not specified | Final |
| 7 | Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology: Guidance for Industry | 23/06/2014 | Office of the Commissioner, Office of Policy, Legislation, and International Affairs, Office of Policy **(US)** | Premarket, Food and Color Additives, Records | Final |
| 8 | Joint MHLW/EMA Reflection Paper on the Development of Block Copolymer Micelle Medicinal Products | 10/01/2014 | Ministry of health labor and welfare and European medicine agency **(JP/EU)** | EMA/ CHMP/13099/2013 | Final |
| 9 | Reflection Paper on Surface Coatings: General Issues for Consideration Regarding Parenteral Administration of Coated Nanomedicine Products | 15/08/2013 | European Medicine Agency **(EU)** | EMA/325027/2013 | Final |
| 10 | Reflection Paper on the Data Requirements for Intravenous Liposomal Products Developed with Reference to an Innovator Liposomal Product Rev 2 | 21/03/2013 | European Medicine Agency **(EU)** | EMA/ CHMP/806058/2009/ Rev. 2 | Final |

**TABLE 2.2**
**Comparison of Regulatory Requirements across Regions [36–38]**

| Sr. No. | Critical Parameter | | |
|---|---|---|---|
| | **US/EU** | **Canada** | **India** |
| 1 | Composition, assay of DS | Composition, identity | Detailed description of individual components, drug loading and entrapment efficiency |
| 2 | PSD and SPAN, particle concentration | PSD | PSD by number, volume and intensity, PDI |
| 3 | Shape and size (aspect ratio), coating thickness, porosity | Morphology | shape, surface properties, state of API in nanostructure |
| 4 | Physical and chemical stability, distribution of API in nanostructure, free and bound drug, structural attributes that relate to function (e.g., lamellarity, core-shell structure) | Stability (chemical reactivity, agglomeration/ aggregation) | Stability of colloids |
| 5 | Structure information, crystal form, Surface properties (e.g., surface area, surface charge, chemical reactivity, ligands, hydrophobicity, and roughness) | Structure information, crystal form, Surface properties (area, charge), water solubility/dispersibility, electrical, mechanical and optical properties | Structure information, crystal form, surface properties (area, charge), water solubility/dispersibility, electrical, mechanical and optical properties |
| 6 | pH, osmolality, viscosity | pH, osmolality, viscosity | pH, osmolality, viscosity |
| 7 | Assay and Impurities | Assay and impurities | Assay and impurities |
| 8 | *In vitro* drug release | *In vitro* drug release | *In vitro* drug release |
| 9 | Sterility, endotoxin levels, and pyrogenicity | Not specified | Sterility, endotoxin levels, and pyrogenicity, residual solvent content |
| 10 | Biodegradability of the nanomaterials and their constituents, compatibility of the nanomaterial relevant to in-use conditions | Catalytic or photo catalytic activity | Manufacturing process and in-process controls, waste disposal method |

There are no harmonized guidance's available for nanoparticles. Currently, generic companies rely on product-specific guidance's to meet the regulatory requirements to get the approval of their generic versions.

## 2.4 CHEMISTRY CONSIDERATIONS

### 2.4.1 Characterization of Nanoparticles

Characterizing nanomedicines involves a complex array of analytical tests to ensure their safety, efficacy, and quality. This process is challenging due to the unique properties of nanoparticles and the stringent regulatory requirements [29].

**TABLE 2.3**
**Parameters Needed to Be Determined and the Corresponding Characterization Techniques [39]**

| Physicochemical Properties | Characterization Techniques |
|---|---|
| Agglomeration state | Zeta potential, DLS, UV-Vis, SEM, Cryo-TEM, TEM |
| Chemical characterization | X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) |
| Chemical state–oxidation state | X-ray absorption spectroscopy (XAS), electron energy loss spectroscopy (EELS), XPS, Mössbauer |
| Concentration | Inductively coupled plasma mass spectrometry (ICP-MS), Ultraviolet-visible spectroscopy (UV-Vis) |
| Crystal structure | XRD, extended X-ray absorption fine structure (EXAFS) |
| Density | Differential centrifugal sedimentation (DCS) |
| Growth kinetics | Small-angle X-ray scattering (SAXS), NMR, TEM, cryo-TEM, liquid-TEM |
| Ligand binding/composition/ density/ arrangement/mass, surface composition | XPS, FTIR, NMR, secondary-ion mass spectrometry (SIMS), ferromagnetic resonance (FMR), thermogravimetric analysis (TGA), small-angle neutron scattering (SANS) |
| Magnetic properties | Superconducting quantum interference device (SQUID), vibrating-sample magnetometer (VSM), Mössbauer, Magnetic force microscopy (MFM), FMR, X-ray magnetic circular dichroism (XMCD), magnetic susceptibility |
| Optical properties | Polarography, electron energy loss spectroscopy (EELS) |
| Particle size, surface characteristics, and morphology | Nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), 3D-tomography |
| Structural defects | HRTEM, electron backscatter diffraction (EBSD) |
| Surface area, specific surface area | Brunauer–Emmett–Teller (BET), nuclear magnetic resonance (NMR) |
| Surface charge | Zeta potential, electrophoresis |

The complexity of nanomedicines necessitates a more sophisticated and comprehensive approach to characterization. As each component of a nanoparticle serves a specific function, it is essential to quantify each component and to evaluate the relationships and interactions between these components in terms of both stoichiometry and their spatial orientation [29].

The overview of parameters needed to be determined and the corresponding advanced analytical techniques used in characterization of nanomaterials is shown in Table 2.3 [38].

### 2.4.2 Stability Studies

Stability studies are required to ensure determination of shelf life. Like all conventional products ICH Q1 guidelines need to be adopted to monitor quality of the product over shelf life [39]. Apart from conventional tests like assay and related

substances, USFDA recommends following attributes specific to nanomaterials that need to be monitored to ensure product quality during shelf life [35].

1. Nanomaterial size and size distribution;
2. Nanomaterial morphology;
3. Self-association (agglomeration/aggregation);
4. Surface charge (e.g., zeta potential);
5. Drug release kinetics;
6. Drug leakage from a nanomaterial carrier;
7. Degradation of nanomaterial (e.g., removal/exchange of surface ligands);
8. Interaction with formulation or container closure (e.g., compatibility, denaturing of proteins);
9. Reconstitution properties of the product; and
10. Solid state properties (e.g., crystal structure).
11. Dilution effect – in-use stability studies

### 2.4.3 Impurities and Contaminants

API and excipients used in the formulation of nanomedicines are the primary source of impurities and contaminants. A complete understanding of chemical, physical, and biological properties of API is critical to understand possible degradation and carry over during formulation development.

Impurities from active substances or solvents get generated during chemical reaction. These impurities are controlled through specifications as per ICH Q3A guidelines [39].

Process impurities coming from API are generally part of specifications. Degradation products generated from the interaction of the drug substance with an excipient and/or immediate container closure system can be estimated using forced degradation studies at early stage of product development. These impurities are controlled according to the ICH Q3B guidelines [39].

ICH Q3D Guidelines deal with inorganic/elemental impurities. Based on toxicity profile, the decision is taken for selection of source and control of these impurities. Heavy metals can be avoided using distilled, demineralized water, and glass reactors during synthesis [39].

The most common residual solvents involved in nanoparticle production are chloroform, dichloromethane, ethanol, methanol, acetone, and N,N-dimethylformamide which need to be monitored and specified according to the ICH Q3C guidelines [39].

The biological contamination of nanomaterials with endotoxins is a common problem. Highly reactive surface of nanomaterial can adsorb a wide range of molecules from the surrounding to reduce their surface-free energy. Endotoxin attaches to nanomaterials through this phenomenon. Endotoxin adheres to hydrophobic surfaces through its lipid moiety and its phosphate group bind to positively charged surfaces of nanoparticles. Endotoxin is a heat-stable ubiquitous contaminant present in almost all chemicals and glassware used in laboratories. Unless specific precautions are taken it can contaminate the nanoparticles during processing. Most importantly, endotoxin cannot be removed by sterilization methods.

The rabbit pyrogen test (RPT) and the limulus amoebocyte lysate (LAL) assay are the most commonly used endotoxin detection methods that are approved by FDA and EMA, and accepted by almost every country [40].

## 2.5 MANUFACTURING ASPECTS

### 2.5.1 Good Manufacturing Practices (GMP) for Nanoparticles

#### 2.5.1.1 API and Excipients

All the excipients used in the formulation of nanoparticles need to be generally regarded as safe (GRAS) listed and for generics within inactive ingredient database (IIG) limits as per regulatory requirement [41]. The physicochemical properties of an API affect the formulation, hence carefully controlling physical properties of API such as input particle size, polymorphism, impurity profiles, water content, residual solvent content can ensure the batch-to-batch reproducibility of the nanoparticles. In-house guidelines should be placed that emphasize robust quality control measures to ensure consistency across API batches.

Excipients may also result in additional pharmacological properties/functions like enzyme inhibition or induction, induction of immune responses. Such excipients should be avoided in the development stage only [30]. The controls on excipients quality from lot to lot is of paramount importance. Adhering to guidelines like ICH Q8 and Q9 ensures excipient variability is managed effectively. Implementing quality control testing, such as Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC), helps to ensure we select the right lot of excipients for manufacturing.

#### 2.5.1.2 Equipment, Facilities, and Process Control

The manufacturing process and equipment's used in the manufacturing of nanoparticles are complex in nature and thus can suffer from scalability issues. The facilities used for manufacturing of nanoparticles require aseptic manufacturing and packaging areas to ensure sterility of the finished products. Thus, adequate in-process process controls must be established to meet stage wise checkpoints during manufacturing. Use of process analytical technology (PAT) and advanced digital solutions such as artificial intelligence (AI) and machine learning (ML) is coming era solution as it enables real-time monitoring of processes during development and manufacturing [30].

### 2.5.2 Process Development

#### 2.5.2.1 Continuous vs. Batch Processing

The batch manufacturing follows a sequential step-by-step approach, In the contrast, continuous manufacturing utilizes a streamlined one-step process that reduces time and minimizes frequent and intensive cleaning procedures. While batch processing offers advantages like on demand manufacturing, step-by-step control to avoid losses and minimize cost involved [42]. However, it suffer from challenges such as extensive documentation, exhaustive cleaning processes, and repetitive validation

requirements [42]. Additionally, batch processing often faces frequent media fill failures leading to delays in the subsequent product manufacturing [31].

On the other hand, continuous manufacturing improves the productivity by being more time-efficient, requiring less cleaning steps, and online controls [43]. It also involves less human intervention that lowers the risk of contamination and reduces need for manpower [43]. The whole process can be fully automated and controlled via computer systems. However, setting up this infrastructure requires substantial costs to ensure smooth operations. Although, continuous manufacturing decreases the likelihood of media fill failures, identifying and addressing the root cause, if it occurs, remains a huge challenge [43]. Technologies such as high shear mixing (HSM), direct and indirect sonication, high pressure Homogenization (HPH), microfluidization, microfluidics, and millifluidics are particularly adopted for continuous processing. This flexibility of configuration makes them more attractive from an industrial standpoint as the manufacturing can be implemented in either mode of operation (i.e., batch or continuous) [31, 42].

Looking at the emergence of AI and ML, it is evident that manufacturing industry will be moving toward continuous processing of nanoparticles.

#### 2.5.2.2 Sterilization and Packaging

Sterilization plays critical role in the products that need to be administered mainly through parenteral routes. Different sterilization techniques are available such as autoclaving, gamma irradiation, electron beam irradiation, aseptic filling, ethylene oxide sterilization to name a few. However, each process suffers from some advantages and disadvantages which directly affect and change the properties of nanoparticles during sterilization [4]. While European agency prefers terminal sterilization process, USFDA is more flexible in terms of methods of sterilization. Validating sterilization is a critical aspect requiring confirmation that complete sterilization has been achieved by assessing D values, which indicate the effectiveness of the sterilization process.

Packaging of nanoparticles is the last stage of the manufacturing process. However, interaction with primary packaging materials can adversely impact the product performance during stability. For example, Glatiramer acetate reacts with silicone coating applied on the glass vials and form impurities in the form of aggregates. Likewise, excipients in nanoparticles can also interact with packaging materials to form adduct. To monitor these interactions, compatibility studies with tubes used in manufacturing, extractable and leachable studies with primary packing materials should be performed during scale-up and pilot manufacturing.

## 2.6 CONTROL STRATEGIES

### 2.6.1 Quality by Design (QbD) and Critical Quality Attributes (CQAs)

Quality by design (QbD) is a systematic approach to pharmaceutical development that emphasizes understanding and controlling the manufacturing process to ensure the final product meets predefined quality criteria. This approach is particularly important in the development of nanomedicines due to their complex nature. Quality

target product profile (QTPP) defines the desired characteristics of the final product, including safety, efficacy, and quality attributes. Critical quality attributes (CQAs) identify the physical, chemical, biological, and microbiological properties that must be controlled to ensure product quality. For nanomedicines, this includes particle size, surface charge, and drug release profile. Risk assessment evaluates potential risks to product quality and identifies critical process parameters (CPPs) that need to be controlled. Techniques like failure mode and effects analysis (FMEA) are often used. Design of experiments (DoE) utilizes statistical methods to systematically investigate the relationships between factors affecting the process and the CQAs. This helps in optimizing the manufacturing process. Control strategy develops a comprehensive plan to monitor and control the CPPs and CQAs throughout the manufacturing process. This ensures consistent product quality. Continuous improvement implements a feedback loop to continuously monitor and improve the manufacturing process based on real-time data and performance metrics [44].

By integrating QbD principles, nanomedicine developers can achieve a more robust and reliable manufacturing process, leading to higher-quality products and smoother regulatory approval.

### 2.6.2 Defining CQAs for Nanoparticle-Based Drugs

Nanomaterials may be sensitive to process conditions and scale-up. Thus, it is important to understand the desired clinical and therapeutic outcomes and which properties, or CQAs, affect such products. Early identification of CQAs can help manufacturers develop and implement appropriate in-process controls [31].

### 2.6.3 Quality Target Product Profile

QTPP of nanoparticles can be defined based on the intended use of the product. It involves defining the dose, dosage form, route of administration, identity, purity, sterility, physicochemical characteristics, drug release kinetics, and PK properties. For generic companies, matching all these properties with that of reference listed drug (RLD) is mandatory in order to have approvable generic product [31, 44].

### 2.6.4 Critical Quality Attributes

Once the QTPP is established, the next step is to identify the key physicochemical and biological properties of the drug product that can affect the product performance. The CQAs of nanoparticles are essential properties that must be controlled to ensure the quality, safety, and efficacy of the final product. These attributes have been already discussed in formulation and process challenges in Sections 2.2.2.1 and 2.2.2.2 [30].

### 2.6.5 Critical Material Attributes and Critical Process Parameters

The fish bone diagram (Figure 2.1) illustrates how CMAs including excipients APIs and environmental factors effect CQAs like particle size morphology and sterility of the drug product. It also highlights effect of critical process parameters like

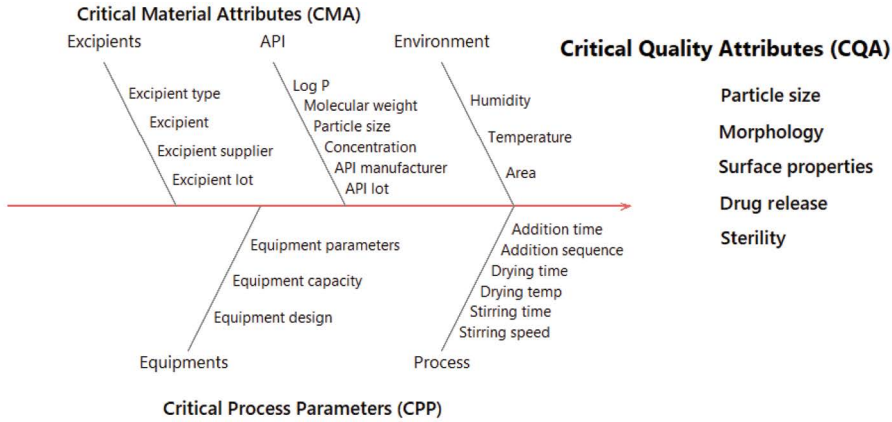


**FIGURE 2.1** Impact of CMA and CPP on CQAs of nanomaterials.

equipment type and its processing parameters during drug manufacturing on the CQAs of the finished product.

### 2.6.6 Risk Assessment

Risk assessment for nanoparticles is usually done in three stages in contrast with conventional formulation where generally two risk assessments are there: initial and final [44].

a. Initial risk assessment: done before experiment starts based on domain knowledge.
b. Updated risk assessment 1: after finalization of prototype formulation and before going to scale up.
c. Updated risk assessment 2: after scaling up of formulation, risks are updated from high/medium to low and control strategy is established.

### 2.6.7 Control Strategy

Control strategy for nanoparticles can be established using knowledge from lab and scale-up batch experiences. The detailed view of control strategy encompassing CQAs and analytical techniques is presented in Figure 2.2.

## 2.7 PRECLINICAL AND CLINICAL CONSIDERATIONS

### 2.7.1 Toxicological Evaluation

Toxicological studies are carried out to determine the potential adverse effects of nanoparticles on human health and the environment [45]. This includes *in vitro* and *in vivo* studies to assess cytotoxicity, genotoxicity, and ecotoxicity. *In vitro* studies include cytotoxicity assays, genotoxicity tests, and oxidative stress assays. *In vivo*

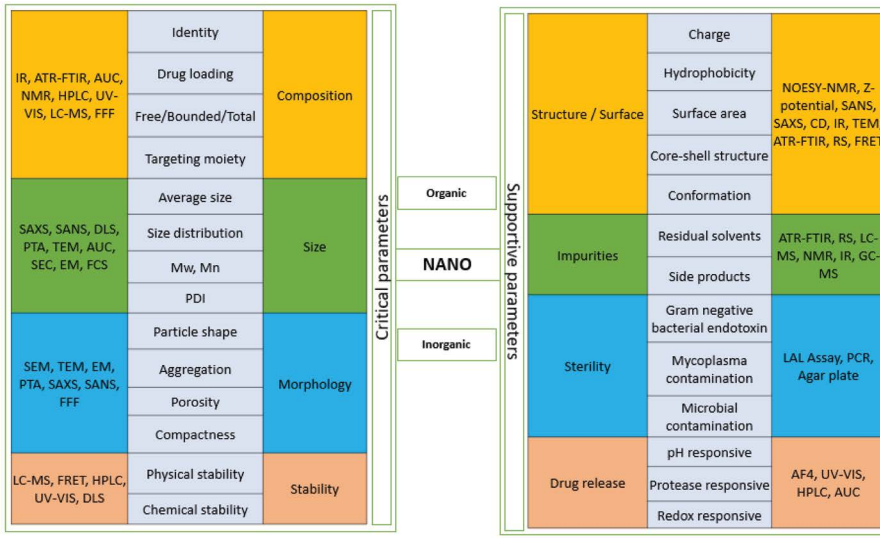


**FIGURE 2.2** Overview of control strategy for CQAs.

studies include acute and chronic toxicity studies, biodistribution and clearance studies, and PK and pharmacodynamic (PD) studies.

### 2.7.2 Biodistribution and Clearance Studies

Biodistribution and clearance studies are essential for understanding how nanoparticles behave in the body, which is crucial for their safe and effective use in medical applications [46]. Techniques like fluorescence imaging, magnetic resonance imaging (MRI), and positron emission tomography (PET) studies are used to track the distribution of nanoparticles *in vivo*. These methods help visualize where nanoparticles accumulate in the body. Evaluation of nanoparticles reaching the target site involves studying the interaction of nanoparticles with biological barriers and their ability to evade the immune system. Organ distribution studies determine the concentration of nanoparticles in various organs (e.g., liver, spleen, kidneys) to assess potential off-target effects and toxicity. Clearance of nanoparticles from the body is characterized by half-life from blood as well as organs where they get accumulated [47].

### 2.7.3 PK Difference

The PK of nanoparticles differ significantly from traditional drug formulations due to their unique properties. Nanoparticles can enhance the absorption of drugs that are poorly soluble in water by increasing their surface area and improving their dissolution rate [48]. Nanoparticles can be engineered to target specific tissues or cells, improving the distribution of the drug to the desired site of action. This targeted delivery can reduce off-target effects and enhance therapeutic efficacy. The size, surface charge, and surface modifications (e.g., PEGylation) of nanoparticles influence

their distribution in the body. For example, smaller nanoparticles tend to penetrate tissues more easily, while surface modifications can help evade the immune system. Nanoparticles can protect drugs from metabolic degradation, increasing their half-life and bioavailability. This is particularly useful for drugs that are rapidly metabolized in their free form. The clearance of nanoparticles from the body depends on their size, shape, and surface properties. Smaller nanoparticles are typically cleared more quickly via renal excretion, while larger ones may be taken up by the RES and cleared through the liver and spleen. These differences highlight the potential of nanoparticles to improve drug delivery and efficacy, but they also underscore the need for thorough PK studies to understand their behavior in the body [49].

### 2.7.4 Safety Issue

Nanoparticles pose significant safety concerns during manufacturing and disposal of the same. The major safety issues arise are from ingestion or inhalation of nanoparticles or coming contact with skin during manufacturing and handling. These exposures can lead to significant toxicity and hence safety regulations need to be put to avoid these exposures. The CMC control strategy should also include guidelines for prevention of exposure of nanoparticles and proper disposal of nanoparticles considering environmental conditions [16].

### 2.7.5 Immunogenicity

The immunogenicity of nanoparticles refers to their ability to provoke an immune response. This is a critical consideration in the development of nanoparticles, as it can impact both the safety and efficacy of the treatment. Immune responses are mainly of two types: innate and adaptive. The innovator companies need to take care of factor that can trigger immune response by modifying surface characteristics or controlling the release [50].

### 2.7.6 Clinical Trial Design

Designing clinical trials for nanoparticles involves several unique considerations due to their distinct properties and potential interactions with biological systems [51]. To proceed for clinical trials of nanoparticles require extensive non-clinical trials data to completely eliminate any safety concerns first followed by PK-PD studies to ensure enhanced efficacy. Clinical trial design for nanoparticles typically will involve the following major points:

- Identification of study design
- Selection of patients
- Selection of dose
- Selection of route of administration
- Identification of primary and secondary end points
- Evaluation of adverse effects
- Risk-to-benefit ratio

Analysis of clinical data is very important to understand the behavior of nanoparticles against placebo or conventional dosage form. Physiological-based pharmacokinetic (PBPK) modeling can also be used for model-integrated clinical study design to minimize the duration of studies or to study drug-drug interactions.

## 2.8 REGULATORY CHALLENGES AND TRENDS

Regulatory guidance on the CMC of nanoparticles is crucial for ensuring the safety and efficacy of products containing nanomaterials. Regulating nanoparticles presents several unique challenges due to their distinct properties and behaviors at the nanoscale [52]. Here are some of the key challenges:

a. Inadequate Knowledge
   There is still a limited understanding of how nanoparticles behave in biological systems and the environment. This makes it difficult to predict their long-term effects and potential risks.
b. Standardization Issues
   The lack of standardized nomenclature, test methods, and characterization techniques for nanoparticles complicates regulatory processes. Consistent and universally accepted standards are essential for accurate assessment and comparison.
c. Risk Profiles
   Nanoparticles often exhibit increased biological activity and unique properties compared to their larger counterparts. These differences can lead to novel risk profiles that are not well understood, posing challenges for safety assessments.
d. Regulatory Frameworks
   Existing regulatory frameworks may not be fully equipped to address the specificities of nanomaterials. Adapting these frameworks or developing new ones to effectively regulate nanoparticles is a complex task.
e. Interdisciplinary Expertise
   Effective regulation of nanoparticles requires expertise from multiple disciplines, including chemistry, biology, toxicology, and materials science. The shortage of trained personnel with this interdisciplinary knowledge can hinder regulatory efforts.
f. Ethical and Legal Considerations
   The unique properties of nanoparticles raise ethical and legal questions, particularly in clinical trials and patient use. Ensuring informed consent and addressing potential public health implications are critical but challenging aspects.

## 2.9 APPROVED NANOFORMULATIONS IN US

Examples of approved nanoparticle-based drugs are presented in Table 2.4.

**TABLE 2.4**
**List of Approved Nanomedicines by USFDA [53]**

| Drug Name/ Brand Name | Company | Indication/Drug Delivery Type | Date of Approval | First Generic Available |
|---|---|---|---|---|
| **Liposomal Injection/Suspension (Lyophilized/Colloidal Solution)** | | | | |
| Amphotericin B/ AMBISOME™ | ASTELLAS | Fungal infections/lyophilized liposomal injection | 08/11/1997 | 12/14/2021 |
| Doxorubicin HCl/ DOXIL™ | BAXTER HEALTHCARE CORP | Ovarian carcinoma, Kahler disease, AIDS-related Kaposi's sarcoma/liposomal injection | 11/17/1995 | 02/04/2013 |
| Daunorubicin and Cytarabine/ VYXEOS™ | CELATOR PHARMS | Newly diagnosed therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC)/ lyophilized liposomal injection | 08/03/2017 | No |
| Bupivacaine/ EXPAREL™ | PACIRA PHARMS INC | Postsurgical local analgesia, regional interscalene brachial plexus nerve block/lyophilized liposomal injection | 10/28/2011 | No |
| Irinotecan/ ONIVYDE™ | IPSEN INC | Metastatic pancreatic adenocarcinoma/liposomal injection | 10/22/2015 | No |
| Amikacin sulfate/ ARIKAYCE KIT™ | INSMED INC | Refractory Mycobacterium avium complex (MAC) lung disease/ liposomal inhalation suspension | 09/28/2018 | No |
| Morphine sulfate/ DEPODUR | PACIRA PHARMS INC | Pain management/liposomal epidural injection | 05/18/2004 (Discontinued) | No |
| Verteporfin/ VISUDYNE | BAUSCH LOMB IRELAND | Treatment of macular degeneration, pathologic myopia/ liposomal injection | 04/12/2000 | No |
| Cytarabine/ DEPOCYT | PACIRA PHARMS INC | Intrathecal treatment of lymphomatous meningitis/ liposomal injection | 04/01/1999 (Discontinued) | No |
| Vincristine/ MARQIBO | ACROTECH | High-grade non-metastatic osteosarcoma and myosarcoma/ liposomal IV solution | 08/09/2012 (Discontinued) | No |
| Patisiran sodium/ ONPATTRO | ALNYLAM PHARMS INC | Polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults/liposomal IV solution | 08/10/2018 | No |
| **Polymeric Nanosuspension (Lyophilized)** | | | | |
| Paclitaxel/ ABRAXANE™ | ABRAXIS BIOSCIENCE, LLC | Metastatic breast carcinoma, locally advanced or metastatic non-small cell lung cancer (NSCLC), metastatic adenocarcinoma of the pancreas/lyophilized nanosuspension injection | 01/07/2005 | 04/12/2022 |

*(Continued)*

**TABLE 2.4** ***(Continued)***
**List of Approved Nanomedicines by USFDA [53]**

| Drug Name/ Brand Name | Company | Indication/Drug Delivery Type | Date of Approval | First Generic Available |
|---|---|---|---|---|
| Sirolimus/ FYARRO™ | AADI BIOSCIENCE | Locally advanced non-operable or metastatic malignant perivascular epithelioid cell tumor/lyophilized nanosuspension injection | 11/22/2021 | No |
| | | **Microspheres (Intramuscular/Periodontal)** | | |
| Risperidone/ RISPERDAL CONSTA™ | JANSSEN | Schizophrenia and bipolar disorder/microsphere IM injection | 10/29/2003 | No |
| Leuprolide acetate/ LEUPRON DEPOT™ | ABBVIE ENDOCRINE INC | Advanced prostatic cancer/ microsphere IM injection | 01/26/1989 | No |
| Naltrexone/ VIVITROL™ | ALKERMES | Alcohol dependence/microsphere IM injection | 04/13/2006 | No |
| Octreotide acetate/ SANDOSTATIN LAR™ | NOVARTIS | Acromegaly/microsphere IM injection | 11/25/1998 | No |
| Triptorelin pamoate/ TRELSTAR™ | VERITY | Advanced prostatic cancer/ microsphere IM injection | 06/15/2000 | No |
| Minocycline hydrochloride/ ARESTIN™ | ORAPHARMA | Adult periodontitis/microsphere periodontal pockets | 02/16/2001 | No |
| | | **Depot Suspensions (Intramuscular)** | | |
| Paliperidone palmitate/ INVEGA SUSTENNA™ | JANSSEN PHARMS | Schizophrenia/1-month IM depot suspension injection | 07/31/2009 | No |
| Paliperidone palmitate/ INVEGA TRINZA™ | JANSSEN PHARMS | Schizophrenia/3-month IM depot suspension injection | 05/18/2015 | No |
| Paliperidone palmitate/ INVEGA HAFYERA™ | JANSSEN PHARMS | Schizophrenia/6-month IM depot suspension injection | 05/18/2015 | No |
| Aripiprazole monohydrate/ ABILIFY MAINTENA KIT™ | OTSUKA PHARM CO LTD | Schizophrenia and bipolar disorder/IM depot suspension injection | 02/28/2013 | No |

*(Continued)*

**TABLE 2.4** ***(Continued)***
**List of Approved Nanomedicines by USFDA [53]**

| Drug Name/ Brand Name | Company | Indication/Drug Delivery Type | Date of Approval | First Generic Available |
|---|---|---|---|---|
| Aripiprazole lauroxil/ ARISTADA INITIO KIT™ | ALKERMES INC | Schizophrenia/IM depot suspension injection | 06/29/2018 | No |
| Cabotegravir/ APRETUDE™ | VIIV HLTHCARE | HIV-1/IM depot suspension injection | 12/20/2021 | No |
| Cabotegravir; Rilpivirine/ CABENUVA KIT™ | VIIV HLTHCARE | HIV-1/IM depot suspension injection | 01/21/2021 | No |
| | | **Depot Gels (Subcutaneous)** | | |
| Granisetron/ SUSTOL™ | HERON THERAPS INC | Chemotherapy-induced nausea and vomiting/extended-release SC injection | 08/09/2016 | No |
| Lanreotide acetate/ SOMATULINE DEPOT™ | IPSEN PHARMA | Acromegaly/SC injection | 08/30/2007 | No |
| | | **Nano Emulsion** | | |
| Estradiol hemihydrate/ ESTRASORB | EXELTIS USA INC | Treatment of moderate-to-severe vasomotor symptoms associated with menopause/topical | 10/09/2003 | No |
| Propofol/ DIPRIVAN | FRESENIUS KABI USA | Anesthetic agent/injection | 10/02/1989 | 01/04/1999 |
| Cyclosporine/ RESTASIS | ALLERGAN | Chronic dry eye/ophthalmic emulsion | 10/10/2003 | No |
| | | **Polymer-Based Nanoconjugate** | | |
| PEG-Aptanib sodium/ MACUGEN | BAUSCH AND LOMB INC | Wet age-related macular degeneration/intravitreal Injection | 09/17/2004 | No |
| | | **Metallic Iron Colloidal Solution** | | |
| Iron sucrose/ VENOFER | AM REAGENT | Treatment of iron-deficiency anemia | 11/06/2000 | No |
| Iron dextran/ INFED | ALLERGAN | Treatment of iron-deficiency anemia | 04/29/1974 | No |
| Sodium ferric gluconate/ FERRLECIT | SANOFI AVENTIS US | Treatment of iron-deficiency anemia | 02/18/1999 | 03/31/2011 |
| Ferumoxytol/ FERAHEME | COVIS | Treatment of iron-deficiency anemia | 06/30/2009 | 01/15/2021 |
| Ferric carboxymaltose/ INJECTAFER | AM REAGENT | Treatment of iron-deficiency anemia | 07/25/2013 | No |

## 2.10 LESSONS LEARNED FROM THEIR REGULATORY JOURNEYS

The ideation behind the concept of these approved nanoformulations is the unmet medical need such as reduction of toxicity (safety), better efficacy (targeting the desired receptor, long circulation time resulting in extended duration of action), and patient compliance. These nanomedicines have been developed on the basis of clinical experience gathered from their simpler versions and using the state-of-art drug delivery science. To get the regulatory approval from various regulatory agencies and enter the market, these products have gone through rigorous non-clinical studies and clinical trials both in healthy subjects as well as patients to ensure safety first.

From Table 2.4, it is evident that developing generic for these nanotherapeutics is not a straightforward task as even after multiple years of approval generic drugs could not get approval though the patents on many of these products are expired. This is largely because it requires innovative approaches to overcome the development, manufacturing, and bioequivalence challenges.

## 2.11 FUTURE PERSPECTIVES

The advent of generic nanoparticles has already happened, presenting challenges for both manufacturers and regulators in demonstrating bioequivalence to innovator products. For example, attempts to replicate the nab-paclitaxel formulation have failed to match its particle size distribution, stability, potency, and physicochemical characterization details, leading to potential safety issues [54].

The overall behavior, PK, and safety profile of nanoparticles result from the interplay of all components, parameters, and spatial composition. There is limited understanding of the relationship between physicochemical properties of nanoparticle and their impact on *in vivo* performance. Conventional preclinical models may not accurately predict nanoparticle distribution, clearance, and toxicity in humans. This is particularly relevant when comparing complex nanoparticle-based drugs with other simple formulations and evaluating generics for approved ones. Bioequivalence cannot be assumed based on similar results in general PK and toxicity studies or simple composition comparisons. Disease models should be used to reflect nanomedicine pharmacology at target sites.

Regulatory agencies must develop comprehensive tests and streamlined approval processes that cover particle characterization, pharmacology, and toxicology. The overall behavior, PK, and safety profile of nanoparticles result from the interplay of all components and parameters. Conventional animal models may not accurately predict nanoparticle biodistribution and toxicity in humans, making it essential to use disease models when possible [55].

Clinical trials can assess short-term toxicity, but long-term effects of nanoparticle accumulation and chronic exposure require ongoing monitoring. Many nanoparticle-based medicines target specific receptors or proteins, such as those expressed by tumors. A personalized approach to treatment, identifying patient subgroups likely to respond, could reduce clinical trial sizes and expedite approval.

Regulatory pathway for nanoparticles is not straightforward as it seems due to multiple challenges involved in the nanoparticle development. At present USFDA and EMA provide guidance for new nanoparticles case-by-case basis. There is a

general lack of standardized guidelines for evaluating nanomedicines as a distinct class of therapeutics [21]. Efforts to establish definitions and guidelines are initial steps toward establishing regulations for nanoparticles. Additional regulations would be enhanced safety and efficacy testing procedures, developing animal or *exvivo* models to improve predictability into humans, develop advanced imaging techniques to study distribution in the body, improvement of facilities that minimizes nanoparticle exposure during handling and manufacturing and involvement of more and more technology in the form of digital tools.

In summary, a thorough understanding of the physicochemical properties and critical parameters of nanomedicine products is crucial for developing generic versions. Regulatory agencies must adapt to the complexities of nanomedicines to ensure that safe and effective treatments reach the market.

## 2.12 CONCLUSION

New nanoparticle-based medicine must overcome several challenges before it can be approved for marketing. These include developing the nanoparticles with the right components and properties, engineering a reproducible manufacturing process, selecting orthogonal analysis methods for adequate characterization, ensuring a favorable pharmacology and toxicity profile, and demonstrating safety and efficacy in clinical trials. Although these challenges are similar to those faced by any new drug, the complexity and multicomponent nature of nanomedicines introduce many additional variables, making it more difficult to control processes and predict behavior in biological systems.

Controlling the impurities and endotoxins in the nanoparticles is big task which needs stringent manufacturing, sterilization, and packaging controls. Selection of right packaging materials is critical to ensure stability of formulation until they reach the consumers.

Nanoparticles being complex systems, need more stringent quality monitoring to ensure minimum failures when scaled at commercial scale. QbD approach should be adopted from the beginning of the development up to the large scale. Adequate control strategy is key to ensure smooth manufacturing process and reduce failures. Adopting AI and ML tools can be beneficial but also challenging to implement for everybody due to involvement of infrastructure and cost.

When it comes to generic nanoparticles, generic company need to follow the product-specific guidance's recommended by regulatory agencies to match qualitative (Q1), quantitative (Q2), and characterization details (Q3) along with PK end-point bioequivalence studies. This seems to be a reasonable approach, but with hidden challenges. It can be seen from the meager number of generic approvals over the last few decades or so. The complexity lies in matching Q3 characterizations and tricky bioequivalence studies. In nutshell, nanoparticle-based drug delivery is need of time and more advancements in the API manufacturing, emergence of novel excipients, advances in manufacturing methods and AI involvement are going to make things more and more complex for generic companies. Generic companies have to think out of the box to overcome the challenges and adopt new technologies to break the difficult barriers of patents.

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# 3 Infrastructure Requirements for Nanomedicine Manufacturing

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## 3.1 INTRODUCTION

Conventional formulations in the form of tablets, pills, capsules, creams, aerosols, suppositories, injectables, etc., were developed in the past for simple molecules that had rapid therapeutic effect for the intended disease [1, 2]. These traditional drug delivery techniques had several disadvantages majorly being the side effects and toxic effects that were due to their lack for target specificity [3]. Apart from these, a higher dose was often required to establish therapeutic effect, and there was a need for controlled drug release [3, 4]. The therapeutic outcome is a result of several factors like the size, chemical composition, hydrophilicity, and ability to bind specific receptor. Lack of solubility leads to poor bioavailability and together with lack of permeability often leads to poor therapeutic outcomes. Hence a stable formulation with drug present in it is essential for drug reaching the target disease and maximum therapeutic effect [5].

The limitations due to the drug properties are the major focus of pharmaceutical industries to be overcome in order. This has been majorly achieved in recent years by shifting from conventional drug delivery system to novel drug delivery systems (NDDS). NDDS focus on developing new ideas, methods, and strategies to overcome the problems of poor solubility, permeability, controlled release, lack of targeting, and assist the active pharmaceutical ingredient (API) to reach the target and exhibit maximum efficacy. NDDS have the ability to tune the formulation characteristics and in turn control the drug properties which help to control and maintain the plasma drug concentration required to show the desired effect for a longer time. This leads to better API performance in terms of safety, efficacy, and patient compliance [6].

### 3.1.1 Liposomes as Nanomedicine for Drug Delivery

#### 3.1.1.1 Liposomes

Liposomes are smectic mesophases with lipid vesicles consisting of bilayers, formed spontaneously in aqueous solutions. These vesicles consist of an aqueous

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core entrapped by one or more bilayers composed of natural or synthetic lipids. These natural lipids are generally biologically inert, non-toxic with low immunogenicity. The hydrophilicity or lipophilicity of the drug determines the location of drug in the vesicles. Those drugs which are hydrophilic tend to accumulate in the aqueous core whereas, lipophilic drugs occupy the lipidic bilayer. Certain drugs have the ability to partition between both the phases, the lipid (within the bilayer) and aqueous core [7].

***Classification of Liposomes:*** The size range developed for liposomal vesicles is in the range of 25 nm to 2.5 μm. Furthermore, they may consist of multiple bilayers or single bilayer. The size of the liposomes and the bilayers present are critical parameters as they can influence the amount of drug encapsulated and circulation half-life. Liposomal systems are thus often classified considering the size and number of bilayers as (1) multilamellar vesicles (MLVs) and (2) unilamellar vesicles. These unilamellar vesicles are further classified into two categories: (1) large unilamellar vesicles (LUVs) and (2) small unilamellar vesicles (SUVs) [8].

***Structural Features and Main Control Factors of Liposomes:*** The organization, structure, and physiochemical properties are often governed by the properties of vesicles like the size, morphology, concentration, and charge of the lipids, as well as the solution properties (such as the ionic strength, pH, temperature) [9]. For pharmaceutical and biomedical applications, the preferred vesicle size encapsulating the drug is often in the range of 50–500 nm [10, 11]. Liposomes below the size range of 150 nm have higher changes of uptake by the cell and can show enhanced permeation and retention effect in the tissues. Liposomes up to size of 100 nm are preferred in cancer biology and targeting the tumor environments. These small-sized vesicles when coated with polymers like polyethylene glycol (PEG) further have the ability to avoid immune system, escape phagocytosis, clearance and exhibit longer blood circulation times [11, 12].

***Conventional Methods for the Preparation of Liposomes:*** Traditionally liposomes were prepared by thin-film hydration technique, wherein the lipids and drugs were dissolved in organic solvents which was subsequently removed. The dried thin film formed was then hydrated with an aqueous phase. However, the use of this technique requires critical control of organic solvent as they may influence the chemical properties of the drug and influence the stability (or toxicity) of the generated nanoformulation [13]. General steps in the development of liposomes using the conventional method are as follows:

- Dissolution of lipids in an organic solvent;
- Drying-down of the resultant lipidic solution from the organic solvent;
- Hydrating the lipid with an aqueous media (followed by agitation/stirring);
- Downsizing (and/or change in lamellarity);
- Post-formation processing (purification, sterilization);
- Characterization of the final nanoformulation product.

#### 3.1.1.1.1 Thin-Film Hydration (TFH) Method (Bangham Method)

The TFH technique, also called the Bangham method is one of the oldest and widely used technique for the preparation of MLVs [14–16]. In this technique, phospholipids are solubilized in an organic solvent, after which the organic solvent is evaporated using rotary evaporator at higher temperature in the presence of inert gases like dry nitrogen or argon stream. Once the lipid thin film is formed, it is hydrated using suitable aqueous medium (buffer) which then hydrates into stacked bilayers. The aqueous medium may consist of polymers, stabilizers, or buffers and appropriate pH may be maintained [13, 17].

Critical process parameters (CPPs) like hydration time and temperature need to be considered. Usually, aqueous hydration can take place for hours depending on the quantity of lipid to be hydrated and the thickness of film formed. Furthermore, this is carried out at elevated temperature around 60–70°C and is generally above the phase transition temperature of lipid. Apart from this mechanical stress in the form of agitation might be required to assist detachment of lipid lamellae for internal vessel surface for sufficient hydration. After this, crude liposomes prepared might be further left for overnight at 4°C to ensure complete hydration. The formed liposomes have heterogeneous size distribution and lamellarity which further need to be homogenized using suitable technique.

#### 3.1.1.1.2 Detergent Removal (Depletion) Method

This method employs use of detergent solution for hydration of lipids [18]. The detergent aids to shield the hydrophobic portion of the lipid and avoids its interaction with the aqueous phase. Following detergent removal, unilamellar vesicles are formed. Some of the commonly used detergents include those having high critical micelle concentration (CMC), such as sodium cholate, Triton X-100, sodium deoxycholate, and alkyl glycoside [13, 19]. There are several techniques to remove the detergent.

- **Dilution Method:** This simple method employs dilution of liposomal solution using suitable aqueous phase or buffer for the removal of the detergent.
- **Detergent Dialysis Method:** In this method, the liposomal solution is dialyzed using a semipermeable membrane against suitable aqueous phase or buffer, wherein the detergent passes through a semi-permeable membrane during dialysis and the vesicles of lipid are formed. This method has several advantages like control over the particle morphology and product homogeneity. Factors that govern these characteristics include the rate of removal of the detergent and lipid – detergent ratio used in formulation. However, potential disadvantages are presence of detergents above specified limits and failure to remove the same.

#### 3.1.1.1.3 Solvent Injection Method

- **Ethanol Injection Method:** This method employs solubilizing the phospholipids in ethanol and rapidly injecting the same into preheated distilled water or buffer. This leads to diffusion of ethanol to the aqueous phase and precipitation of the lipid molecule. The subsequent formation of the bilayer planar fragments encapsulates the aqueous phase. Finally, the ethanol

evaporation favors the fusion of the lipids' fragments and the successive formation of closed unilamellar vesicles [20, 21].

- Material parameter that plays a vital role is the volume of ethanol. It is possible to develop homogeneous SUVs by limiting the concentration of ethanol to 7.5% in the whole formulation. Furthermore, controlling the rate of injection of ethanol can have impact on the size of liposomes formed. This can be achieved using elevated temperatures or reduced pressure for evaporation and removal of solvent. Injecting the ethanol solution rapidly in aqueous phase can lead to a heterogeneous population of MLVs being formed. The advantage of ethanol injection technique is development of both SUV and LUV [21].
- **Ether Injection Method:** Here the phospholipid is solubilized in ether solvent and injected into the aqueous phase. This is followed by heating the above solution to 55–65°C under reduced pressure to remove ether. In this method LUVs are formed [22, 23]. In this technique it is comparatively easy to remove ether as compared to ethanol. However, the use of higher temperature can lead to instability in thermolabile drugs.

#### *3.1.1.1.4 Reverse-Phase Evaporation Method*

Briefly, during preparation of liposomes by this method the phospholipids are solubilized in organic solvent that leads to formation of inverted micelles. Addition of aqueous phase to it leads to rearrangement of lipids at the interface of oil and water and form w/o microemulsion. This is followed by emulsification using mechanical or sonication method. The organic solvent is removed by using a rotary evaporator under reduced pressure and elevated temperatures, if required. The removal of organic phase in a slow gradual way favors the removal of organic solvent leading to disruption of inverted micelles and formation of LUVs. This method has the advantage of encapsulating large volumes of aqueous phase in the core. Furthermore, this process is difficult to scale at industrial levels. Also, large molecules like proteins, peptides, oligonucleotides may undergo a conformational change due to the mechanical stress and exposure to organic solvent leading to protein denaturation, breakage of DNA strands [24–26].

##### *3.1.1.1.4.1 Downsizing of Liposomes*

a. ***Sonication:*** Sonication method employs use of high energy using tip of probe sonicators or by use of bath sonicators. The amplitude of sonication and the time of sonication are critical as it impacts the properties of liposome and drug encapsulation. Use of probe sonication using titanium tips can cause metal impurities to be introduced in the liposomes and also this method becomes unsuitable for protein or sensitive compounds.
b. ***Microfluidizer:*** Microfluidizer technique employs high-speed collision and mixing of two separate streams of fluid under high pressure. Two streams collide in a mixing chamber and the pressure is in the range of 0–200 bar. The temperature of the system is effectively controlled using thermal jackets. After mixing and the formation of liposomes, the size reduction occurs when the liposome passes through the orifice several times [27].

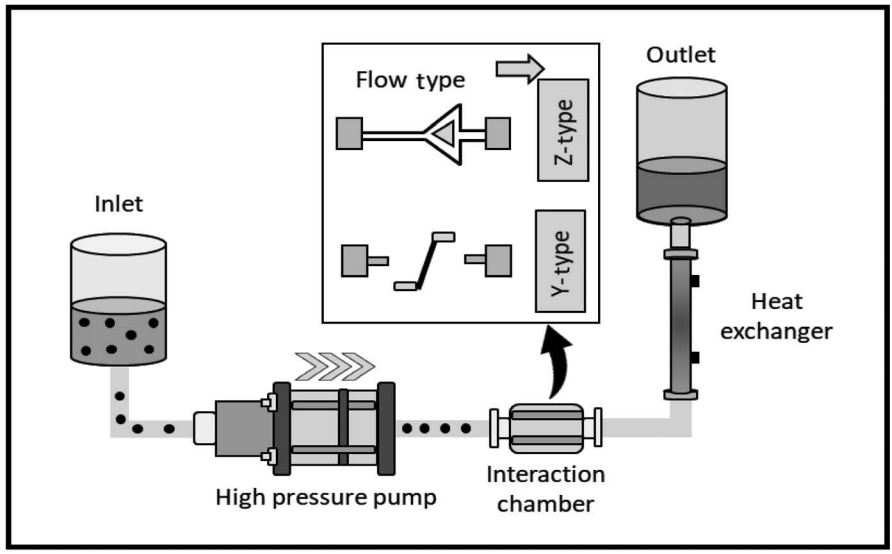


**FIGURE 3.1** Schematic representation of microfluidizer technology for size reduction of liposomes.

Microfluidizers are used in large scale and sterile production of liposomal systems. Schematic representation of working and downsizing of liposomes by microfluidizer is given in Figure 3.1.

CPPs involved during manufacturing of liposomes by microfluidizer technology includes flow rate ratio, pressure, temperature, and duration of drug loading.

c. ***Homogenization:*** In this method fluid beam is passed through an orifice under high pressure and it is collided with stainless steel wall. The liposome suspension is continuously pumped through the homogenizer system, where high pressures are generated to downsize lipid vesicles. During homogenization various factors that govern the size of liposomes include homogenization pressure applied, duration or cycles of homogenization, and temperature of the process [28]. Schematic representation for high-pressure homogenization in size reduction of liposomes is mentioned in Figure 3.2.

d. ***Extrusion:*** Extrusion technique employs use of polycarbonate (PC) membranes having pre-defined pore size through which liposome phase passes. The overall process takes place under pressure and in the presence of nitrogen inert environment. The size of liposomes and their downsizing is governed by the pore size of PC membranes which can generate liposomes of size as low as 25 nm and as large as 1000 nm. The liposomes are extruded above the phase transition temperature (Tc) of the lipids and the temperature can be controlled using thermobarrel of the extruder [29, 30]. Schematic representation of membrane extruders is given in Figure 3.3.

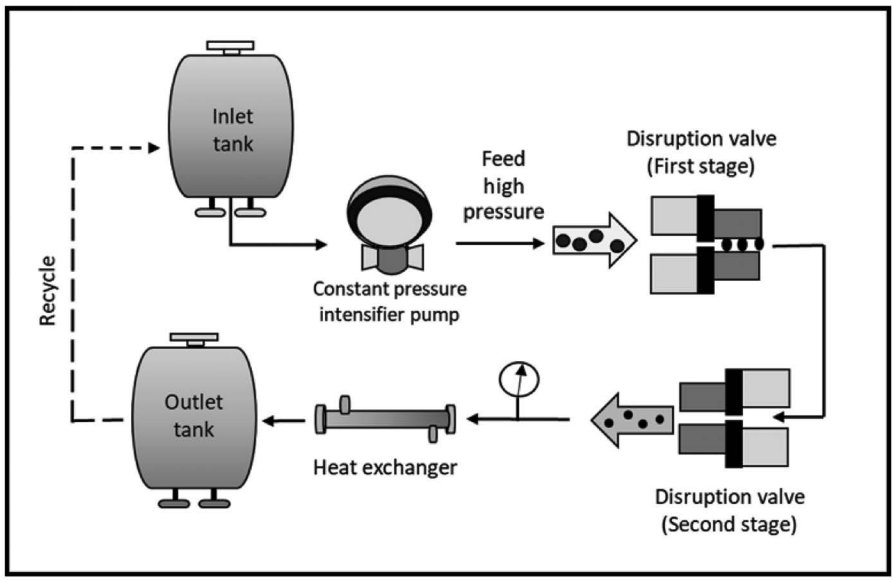


**FIGURE 3.2** Schematic representation of high-pressure homogenization (HPH) for size reduction of liposomes.

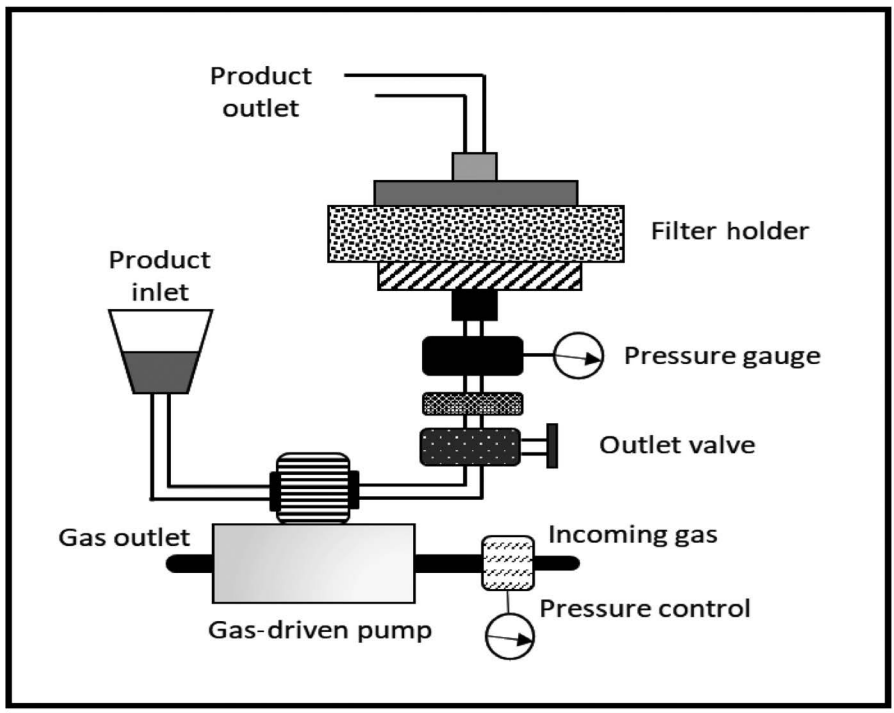


**FIGURE 3.3** Schematic representation of extrusion technique for size reduction of liposomes.

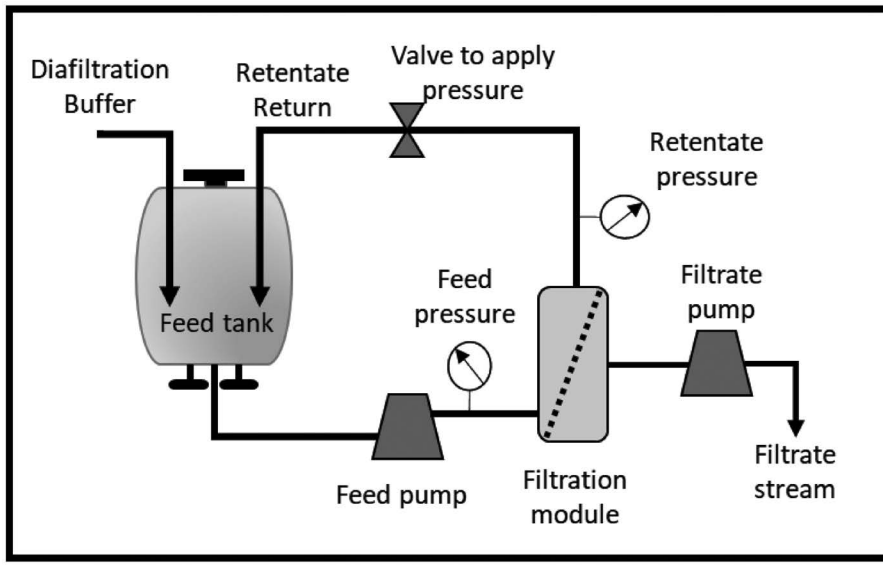


**FIGURE 3.4** Schematic representation of diafiltration process.

*3.1.1.1.4.2 Purification of Liposomes Nanoformulations* Generally, the lipophilic drugs are encapsulated in the bilayer or the aqueous core, however those present in the external environment need to be purified using suitable technique. The purification of liposomes and removal of these unencapsulated drug are usually achieved using techniques like ultra-filtration, ultra-centrifugation, dialysis, and (size exclusion, gel-permeation, or ion-exchange) chromatography.

Popular techniques include diafiltration (Figure 3.4), or tangential flow filtration for purification of liposomes, removal of detergents, unencapsulated API, etc. Critical factors involved during tangential flow filtration for purification of liposomes include membrane size, membrane type and cutoff, surface area of membrane, and diafiltration cycles volume [31, 32].

Furthermore, the residual solvents present need to be removed as its presence may cause destabilization of liposomes. These organic solvents are generally removed under reduced pressure in rotary evaporator. Also, certain unsaturated lipids are sensitive and may undergo oxidation. This can be avoided by maintaining an inert temperature and carrying the process in the presence of inert gases like nitrogen and argon. It is also essential to remove heavy metals and protect the labile formulations from light to avoid metal-induced oxidation or photo oxidation. This is achieved using chelators like EDTA and use of light-resistant containers. Anti-oxidants like alpha-tocopherol or butylated hydroxytoluene are added to minimize lipid oxidation processes [13, 16].

*3.1.1.1.4.3 Sterilization of Liposomes* Many liposomal formulations are administered via parenteral route and hence it is required that these formulations are free of viable microorganisms (such as bacteria, fungi, and spores) Appropriate sterilization

technique needs to be employed taking into consideration the stability of liposomes. Various techniques like steam heating (autoclaving), ultraviolet and gamma ionizing irradiation, chemicals, and filtration methods are employed to ensure the formulation and other materials are controlled within the specified limits of the regulatory guidelines. Higher processing and sterilizing conditions may impact the physicochemical characteristics of the formulation and hence it remains a critical challenge during the development process of liposomal formulation [33].

Autoclaving process employs use of saturated steam at pressure sufficient to cause hydrolysis of proteins in the microorganisms. The autoclaving temperature which is generally above the phase transition temperature of lipids can cause alterations in properties of liposomes leading to aggregation, degradation, hydrolysis, and oxidation. It can also lead to structural phase transitions leading to drug leakage at higher temperatures during autoclaving. This may be a limiting factor for using this technique for sterilizing certain liposomal formulations.

Apart from this other technique to sterilize liposomes is using gamma ionizing irradiation that has a high-energy ionizing power (and then a strong penetration capacity). In this technique the lipid may undergo degradation due to peroxidation (unsaturated lipids) and their fragmentation. UV radiation-based sterilizing is not used as they are non-ionizing and have poor penetration capacity and sterilize the liposomes.

Another technique is using ethylene oxide (chemical) sterilization that uses ethylene oxide gas. Ethylene oxide can cause alkylation of the side chains of DNA, RNA, and enzymes causing alterations in the normal metabolism of microorganisms leading to loss of multiplication of microorganisms and their death. Major limitation of use of ethylene oxide is its explosive nature, along with its toxic, carcinogenic, and mutagenic character of its residues. Hence this method is not used for formulation sterilization.

Sterilization by filtration is one of the most widely used technique controlling microorganisms and developing sterile formulation. The method is time consuming depending on the scale of the batch. Sterile filters consist of disposable filtration unit consisting of an aseptic bacterial-free membrane (0.22 μm) or depth filters that assist in the removal of the microorganisms present in the formulation. However, downsizing of liposomes needs to be done below 200 nm for effectively removing microorganisms. Further size above 200 nm can lead to blocking of filter pores and ineffective filtration. This can lead to product loss, drug loss, and poor entrapment of the drug due to leakage.

Aseptic manufacturing is another way to develop sterile formulations. The whole manufacturing process from preparation to filling is carried out in a controlled sterile environment (class A environment). All the instruments and equipments present in the area are sterilized. Multiple stages associated with this manufacturing process make it complex and expensive. Limitations associated with this method can be lack of evaluation of sterility levels and risk of contamination associated with raw materials that are not properly sterilized.

With the drawbacks associated with the conventional approaches of sterilization it becomes essential to ensure sterility of liposomes in an effective and cost-effective manner [33]. Recently use of super critical carbon dioxide (SC-$CO_2$) technology has gained prominence as an effective alternative for liposome formulation sterilization [34]. SC-$CO_2$ technology allows to sterilize the liposomal nanoformulations in a

single step. However, these approaches require further investigations and optimization using the standard biological indicators.

***Manufacturing of nanomedicine requires specialized infrastructure to ensure safety, identity, strength, purity, and quality of pharmaceutical formulations.***

### Key requirements include

a. ***Cleanroom Facilities***
Cleanrooms are facilities in which the microbial bioburden is controlled as per the regulations which help to control and prevent the contamination of formulation. Compromising with sterility can have serious implications on the safety, identity, strength, purity, and quality of the drug product. The level of airborne particle and other contaminants are controlled in cleanroom facilities using specialized ventilation and filtration units. Cleanroom facilities are critical for nanomedicine manufacturing to ensure the purity and integrity of the products. General requirements for designing the cleanroom facilities are mentioned below:

b. ***Particle Control***
It is essential that cleanrooms monitor and control the levels of viable and non-viable particles which are airborne, as even small particles can contaminate nanomedicine products. HEPA filters that generate high-efficiency particulate air (HEPA) are installed in the required cleanroom areas that help to minimize the particle presence in the area which limits the contamination by viable and non-viable particulates. The limits for viable and non-viable particles to be present in the clean room are defined in various regulatory guidances. ISO designation for the levels of particulates in different area is defined in Table 3.1.

**TABLE 3.1**
**Air Classifications[a]**

| Clean Area Classification (0.5 um particles/ft³) | ISO Designation[b] | >0.5 µm Particles/m³ | Microbiological Active Air Action Levels[c] (cfu/m³) | Microbiological Settling Plates Action Levels[c,d] (diam. 90 mm; cfu/4 hours) |
|---|---|---|---|---|
| 100 | 5 | 3,520 | 1[e] | 1[e] |
| 1000 | 6 | 35,200 | 7 | 3 |
| 10,000 | 7 | 352,000 | 10 | 5 |
| 100,000 | 8 | 3,520,000 | 100 | 50 |

[a] All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.

[b] ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle concentration is equal to Class 100 and approximately equals EU Grade A.

[c] Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological action levels due to the nature of the operation or method of analysis.

[d] The additional use of settling plates is optional.

[e] Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

Table 3.1 summarizes clean area air classifications and recommended action levels of microbiological quality [35].

Considering the criticality of the limits, the production facility has two categories of sterile areas.

c. ***Critical Area***

Critical area is the most important area in the manufacturing process also named as Class 100 (ISO 5). All the materials, including drug product, packaging materials, and equipments, are involved with direct exposure with the drug product and hence need to be sterile. Filling of the product takes place in this area and any contamination during this stage in the product is difficult to be removed in the subsequent stage. Furthermore, any non-viable particulates need to be controlled as they can serve as vehicles for microorganisms and contaminate the product [36].

***Supporting Clean Areas***

Supporting clean areas are regions where nonsterile components, formulated products, in-process materials, equipment, and container/closures are prepared, held, or transferred. These areas have less control over the particulate control but are effective enough to control the level of particle contaminants in the final product. Any bioburden or particulates are removed or subsequently sterilized in the critical area.

FDA specified different class according to the acceptable particulate levels in each class. It is necessary that the area adjoining the accepting processing line has a minimum of Class 10,000 (ISO 7) standards (see Table 3.1) under dynamic conditions. Generally, these areas are Class 1,000 (ISO 6) that are adjoining the aseptic filling room at Class 100 (ISO 5). Less critical activities like equipment cleaning are performed in Class 100,000 (ISO 8) [37, 38].

d. ***Temperature and Humidity Control***

Certain materials are temperature and moisture sensitive and hence it is essential to control and monitor temperature and humidity levels during the manufacturing process. APIs that are hygroscopic can absorb moisture which can lead to incorrect weighing and impact the assay of the drug product. Hence manufacturing rooms are equipped with HVAC systems having automated controls to maintain temperature and humidity set points within tight tolerances. The temperature and humidity are constantly monitored using validated sensors in both critical areas and less critical areas [38, 39].

e. ***Pressure Differentials***

All the different areas in the manufacturing plant have pressure differential as per required standards, to control the flow of air and associated particulates with it. As we move towards the Class 100 area from previous subsequent areas the area pressure increases. This pressure cascade helps to avoid the entry of any viable or non-viable particulates in the subsequent area. This is pressure differential is essential as when the two rooms are opened, the air flows from a higher-class room to the lower-class room preventing contaminants from moving into the higher-class room. ISO 14644-4 recommends of pressure differential from room to room of 5–20 Pascal. In the

case of potent pharmaceutical APIs there is a negative air pressure. Such a pressure differential cascade is maintained to avoid flow of air from lower area to higher area. This differential can be measured using analog devices like Magnehelic gauges or with digital monitoring systems [40–42].

f. ***Cleaning and Sanitization***
Periodic cleaning and sanitization of clean rooms is essential to control and avoid unnecessary bioburden within the cleanrooms. With same sanitizing solutions being used there are chances of resistance being developed in the microorganisms and hence different solutions are used in rotation to avoid the same. Post sanitization the contact time is defined after which the work can be initiated. Contact time defines the time for which a sanitizing solution needs to stay wet on a contact surface, in order to be effective against the microorganism. Different sanitizing solutions have different contact time [43, 44].

g. ***Personnel Training, Qualification, and Monitoring***
Personnel without improper training can be the greatest source of viable and non-viable particulates. Hence personnel need to be properly trained on SOP and strictly follow the all procedures from the entry to exit. Advanced systems use specific personnel access via card or biometric controls. All the areas require the levels of sterility to be maintained and hence validated steps have been outlined for gowning procedures. Personnel need to be trained to avoid direct contact with sterile products, containers, closures, or critical surfaces with any part of their gown or gloves. Furthermore, rapid movement of personnel is restricted as it can lead to creation of air turbulence in the critical area and influence the levels of particulate matter. It is necessary to monitor the levels of bioburden after exit of each personnel. Quality control (QC) personnel can collect samples as monitoring points of personnel from different areas of the gowning like the gloves, hands, forearms, and uniforms from personnel working in the area of aseptic filling room [45]. This is done using sterile media plates and observing from growth of microorganisms [46].

*Training and Certification Programs*: Apart from other requirements mentioned above, the personnel working in specialized laboratories and handling critical instruments and materials can be directed for concerned training and certification programs so that they are equipped with necessary skills to tackle the challenges associated with nanomaterials and biologics manufacturing. Apart from these training programs can cover a wide range of subjects like laboratory safety, chemical handling, and emergency procedures [46].

h. ***Material Compatibility***
The construction of the cleanroom facilities like panels, floorings, furnitures, etc., needs to comply cleanroom protocols. It should be ensured that non-shedding materials are used that can be easily cleaned and bear resistance to chemicals and disinfectants [47].

***Monitoring and Control Systems:*** All the cleanrooms are well equipped several monitoring and control systems that continuously monitor several critical parameters like particle counts, temperature, humidity,

and pressure differentials. These systems are equipped with necessary alarms and alerts notify the personnel of any deviations from the specified limits [48].

***Validation and Certification:*** Regulatory agencies demand validation and certification of cleanroom and its equipments to ensure they meet the necessary pre-requisites of regulations. This involves rigorous testing and documentation of performance parameters [49].

The safety and efficacy of the nanomedicine can be ensured only when all the sterile requirements and facilities meet the stringent standards to minimize contamination risks and maintain product quality.

***Specialized Manufacturing Equipment:*** Development of nanoformulations requires advanced and high-precision equipment like homogenizers, microfluidic devices, and other nanofabrication tools.

***Nanoparticle Characterization Tools***: The developed nanoformulation requires extensive characterization to precisely outline the physicochemical characteristics like the size, shape, surface charge, and composition of nanoparticles. Use of equipments and instruments like electron microscopes, atomic force microscopes, and dynamic light scattering systems, are crucial for QC [50].

***Specialized Laboratories:*** Apart from the above facilities mentioned, dedicated laboratories equipped with biosafety cabinets, fume hoods, and chemical storage facilities are essential to safely handle hazardous materials.

- *Biosafety Cabinets:* In pharmaceutical and biopharmaceutical industry certain products like biologics need special handling and care. Such a secured, contained, and sterile environment is provided by biosafety cabinets. It protects the researchers from such dangerous materials by avoiding cross-contamination and allowing filtered air to flow via HEPA system through the cabinet [51].
- *Chemical Fume Hoods*: Certain materials like solvents, chemicals, or products during synthesis may generate fumes that are hazardous in nature. Hence such materials are handled under fume hoods which allow exhaust of fumes, vapors, and aerosols without affecting the individual to come in contact with it [52].
- *Biological Safety Level (BSL) Facilities*: These biocontainment facilities isolate biohazards like microorganisms, biologics, or other hazardous materials. Specialized containment measures, personnel protective equipment (PPE), and waste disposal systems are provided to ensure the safety of researchers and prevent the release of hazardous agents into the environment [53].

***Quality Control and Testing Facilities:*** Laboratories equipped with analytical instruments for assessing the stability, purity, and potency of nanomedicines throughout the manufacturing process are essential for meeting regulatory standards. These laboratories must have state of the art facilities such that quantitative and qualitative parameters, physicochemical characteristics, biological properties, and other analyte characteristics are precisely estimated. The advanced instrumentations need to characterize according to the product being developed and requirements of the project [54].

***Regulatory Compliance Infrastructure:*** Systems and processes for adhering to regulatory guidelines and good manufacturing practices (GMPs) are critical to ensure product safety and efficacy and facilitate approval for clinical use.

*Data Management and Safety Protocols*: Research and innovative ideas are the most valuable assets for a pharmaceutical company. These ideas need to be documented and protected as lot of time and money might have been spent to execute these ideas. Any experimental work done needs to be documented and must comply strictly with data management and safety protocols to ensure the integrity of research data and other sensitive information. Dedicated IT team must work to secure data storage, access controls, and adherence to regulatory guidelines for data handling and reporting [55].

***Scale-Up Capabilities:*** Infrastructure for scaling up production from laboratory-scale to commercial-scale manufacturing, including larger manufacturing vessels/reactors with required automations for precise controls of fluid transfers and reaction mixtures, automated production lines, and logistics systems, is necessary for mass production of nanomedicines.

***Waste Management Systems:*** Proper waste disposal systems for handling hazardous materials and by-products generated during manufacturing are essential to minimize environmental impact and ensure compliance with regulations [56].

***Research and Development Facilities:*** Facilities for ongoing research and development to innovate new nanomedicines, improve manufacturing processes, and address emerging challenges in the field are essential for long-term success.

Overall, a combination of state-of-the-art facilities, specialized equipment, stringent QC measures, and regulatory compliance infrastructure is required to support the manufacturing of nanomedicines effectively.

Advanced equipment is essential for the precise manipulation, characterization, and production of nanomedicines. Production of nanoformulation at industrial scale requires advanced instruments and equipments for large-scale manufacturing and their continuous evaluation. Different nanoformulation and colloidal systems are developed in R&D which need to be scaled at industrial level. Synthesis of nanoparticle with precise control over size, shape, and content can be achieved using advanced techniques like chemical vapor deposition, sol-gel synthesis, and nanoprecipitation. Apart from this high-pressure homogenizers, microfluidic instruments are available that allow precise control over size, homogeneity, and shape of the particle or vesicle being formed [57–61].

- *Drug Encapsulation and Delivery Systems:* Liposomes are hollow particles bounded by a lipid membrane, hold promise as drug delivery systems. Here are two main techniques for encapsulating drugs in liposomes:
  - *Passive Loading:* Passive loading techniques allow to incorporate both lipophilic drug and hydrophilic drug. Generally, the lipophilic drugs that are solubilized in an organic solvent and evaporated to form thin film, tend to reside in the lipidic bilayers of the liposomes after hydration [62]. However, the hydrophilic drugs are solubilized in the aqueous

phase used for hydrating the thin film of lipid formed. After hydration and formation of the liposomes these hydrophilic drugs remain inside the aqueous core of the liposomes [63]. The encapsulation efficiency varies (5–20%) based on hydration conditions and drug properties. The unloaded drug remains in the surrounding of the aqueous medium and may require additional removal techniques.

- *Active Loading:* Another technique used is active loading technique wherein different methods like generation of pH gradient or ion gradient is used to force drug loading inside the liposomes. Using this technique higher drug concentrations can be loaded and encapsulated [64].

- *Biological Assay Platforms*: The API along with nanomedicines developed is subjected to high-throughput screening platforms and automated assay systems to assess the biological activity, toxicity, and efficacy of developed formulations. Evaluation of these medicines in cellular and animal models helps to better assess the advantages of developed formulation for their biological activity, efficacy, and safety of nanomedicine formulations. Different approaches like cell-based assays, animal studies, *in vitro* toxicity testing, immunological assays, and assessment of pharmacokinetics and biodistribution are adopted to evaluate the same [65, 66].
- *In Vivo Imaging Systems:* After the development of nanomedicines, like liposomes, these formulations are subjected to several *in vitro* and *in vivo* studies. The distribution of the developed formulation can be observed using non-invasive techniques like observations under fluorescence microscopy, confocal microscopy, and *in vivo* imaging modalities (e.g., positron emission tomography, magnetic resonance imaging). These techniques aid to visualize and track nanoparticles in biological systems. They can further assist in qualitative understanding of the biodistribution, pharmacokinetics, and targeting efficiency of nanomedicines *in vivo.* These techniques help to understand the science better and provide a strong scientific support during translation from lab to clinical applications [67, 68].
- *Nanoparticle Characterization Tools:* These tools play a critical role in evaluating the quantitative and qualitative aspects of the developed nanoparticles. Different physicochemical parameters like size, shape, surface charge, and composition can be determined. Here are some key nanoparticle characterization tools:
  - *Transmission Electron Microscopy (TEM)*: TEM assists in obtaining high-resolution images of nanoparticles at an atomic scale. It helps to visualize nanoparticle morphology, size distribution, and internal structure.
  - *Scanning Electron Microscopy (SEM)*: As compared to TEM, SEM aids in capturing the surface characteristics of nanoparticles at lower resolutions. During liposome imaging, cryo electron microscopy is used, as these lipid vesicles are sensitive to the exposed electron beam for imaging. Cryo-section provides critical internal insights on the observed cross section of the developed liposomal formulation. It is useful for analyzing nanoparticle shape, surface morphology, and aggregation behavior [69–71].

- *Atomic Force Microscopy (AFM)*: AFM is a versatile imaging technique that can visualize nanoparticles with nanometer-scale resolution. It provides information on nanoparticle size, shape, surface roughness, and mechanical properties [72].
- *Dynamic Light Scattering (DLS)*: DLS measures the Brownian motion of nanoparticles in suspension that is correlated with the intensity of the scattered light to determine their relative hydrodynamic size distribution. It is commonly used to analyze the size distribution, polydispersity, and stability of nanoparticles in solution [73].
- *Zeta Potential Analyzer*: ZP estimates the electrophoretic mobility of the nanoparticle present. Since this parameter is dependent on the charge present on the particle, it helps to estimate the same. Since charged particles repel each other, understanding of this parameter helps to understand the stability of the developed nanoformulation. It also provides information on nanoparticle stability, colloidal behavior, and surface charge interactions [74].
- *X-Ray Diffraction (XRD)*: As each material whether in nano or microscale has characteristic crystalline arrangement, it is essential to understand the same for the developed nanoformulation. XRD using Bragg's equation helps to analyze the crystalline structure and provide information on the phase composition of nanoparticles. Extensive internal details like nanoparticle crystallinity, crystal size, and lattice parameters can be known using XRD [75].
- *Fourier Transform Infrared Spectroscopy (FTIR)*: FTIR is widely used during the developmental phase of the product. It provides insight on the chemical composition and functional groups present on the surface of nanoparticles. Furthermore, drug stability, drug interaction with the excipients during binding, coating, etc., can be evaluated [76].
- *UV-Vis Spectroscopy*: As each drug has its own absorbance characteristic, this technique is used to estimate the optical properties of nanoparticles, including absorbance and extinction spectra. It provides information on nanoparticle concentration, size, and surface plasmon resonance [77].
- *Raman Spectroscopy*: This technique is the most reliable and widely used technique that provides extensive information on the chemical composition and molecular structure of nanoparticles. It is useful for analyzing nanoparticle surface functionalization, chemical bonding, and impurities [78].
- *Nuclear Magnetic Resonance (NMR) Spectroscopy*: NMR spectroscopy is used to analyze the structure, composition, and dynamics of nanoparticles at the atomic level. It provides information on nanoparticle composition, molecular mobility, and interactions with surrounding molecules and also the lamellarity of the liposomes [79]. It can also serve as tool to estimate the drug encapsulated in liposomes using technique of 1-H NMR [80]. Use of these analytical tools along with sound scientific knowledge helps to understand the properties and behavior of nanoparticles which can be further used to optimize their synthesis processes, and develops effective nanomedicine formulations for various therapeutic applications.

Once the developed formulation is scaled for large-scale manufacturing, several QC and testing need to be done to ensure the developed formulation complies with the expected quality attributes and fulfill the requirements of safety, efficacy, and consistency of the products. Dedicated QC facilities at industrial level are required to meet these criteria. Here are key aspects of such facilities:

- *Physical and Chemical Testing*: Various physical and chemical tests are conducted to assess the properties of nanomedicines, including particle size distribution, surface charge, drug-loading capacity, drug-release kinetics, stability under different conditions (temperature, pH), and chemical composition [81].
- *Sterility Testing*: Sterility testing of parenteral and ophthalmic products is essential as they directly gain access inside the body via blood circulation. It is essential to ensure that these products are devoid of microbial contamination. Depending on the regulatory guidelines and pharmacopeial monograph requirements the samples are tested against specific species of bacteria, fungi, and other microorganisms and tested for their presence or absence [82].
- *Validation Studies*: QC facilities conduct necessary validation studies that ensure the processes are robust, reproducible, and capable of producing high-quality nanomedicine products. This includes validation of analytical methods, equipment performance, and process parameters [83].
- *Regulatory Compliance:* Regulatory agencies must ensure that the QC labs adhere to the guidelines laid by regulatory agencies of respective countries where marketing is intended and the manufacturing plant follows GMPs during product development. This includes documentation, record-keeping, and reporting of QC data [84].
- *Quality Assurance (QA) Systems*: The QA department in the pharmaceutical industry plays a crucial role in ensuring that products meet required quality standards. Here are some key responsibilities of QA professionals:
  - *Monitoring Warehousing Practices*: QA has the role of maintaining and assuring proper storage conditions for API, excipients, packaging materials, containers, and labels. Ensuring required storage conditions is essential to comply as per regulatory standards and expectations [85].
  - *In-Process Quality Assurance (IPQA):* The complete manufacturing process from raw material storage to final product dispatch is monitored and overseen in presence of QA team or personnel. They ensure that paperwork has been done properly and are received in the expected physical and chemical condition. The IPQA has the additional role of collecting in-process manufacturing samples and analyze the same [86].
  - *Process Monitoring and Controls:* QA carriers the critical role to ensure that during the product manufacturing necessary steps employed follow validated procedures and standard operating protocols (SOPs). They critically observe for any deviations observed and find the root cause of the same. The QA analyzes the root cause and ensures the same is not repeated in the future.
  - *Batch Record Review:* Each manufacturing process is critically documented with each steps mentioned in detail. Necessary documentation

is done and records are kept in hard copies and softcopies. The batch records holding vital information like process timing, ingredients, environmental conditions, etc., are evaluated by the QA. If any data integrity issues are found, QA takes corrective action.

*3.1.1.1.4.4 Scale-Up of Liposomal Formulations – Infrastructure Requirements*
Scale-up capabilities are essential for nanomedicine manufacturing to transition from laboratory-scale production to commercial-scale production. Important material and process parameters to be considered during nanomedicine manufacturing are listed in Table 3.2. Here are key aspects of scale-up capabilities:

- *Process Optimization:* Prior transferring the developed formulation to industrial scale it is essential that the product is optimized and the manufacturing process is efficiently reproducible and of required quality. For

**TABLE 3.2**
**Important Material and Process Parameters to Consider during Nanomedicine Manufacturing**

| |
|---|
| Vessel shape (top and bottom of vessel) to ensure cleaning and to check drainability /recovery |
| Stirrer design – Numbers and shape of impeller (flow pattern required for mixing/homogenization of bulk) |
| Stirring volume |
| Sampling volume |
| Temp sensing volume |
| Process temperature |
| Temp control systems (Jacket requirement/Jacket type) |
| CIP (coverage by spray ball) |
| SIP |
| PHT |
| Air filter and vent filters |
| Safety valve (chamber and jacket) |
| Pressure gauge |
| Flush bottom valve |
| Bulk addition provision (charging port/handhole/manhole) |
| Operating system (HMI/PLC) |
| Requirement of load cell/dipstick as per process requirement |
| Light fitting and feasibility of maintenance of light |
| All activities are manual |
| Temperature monitoring/maintenance manual |
| Stirring monitoring/maintenance manual |
| Transfer of bulk manual |
| Manual CIP/SIP |
| No alarm for any accessories related malfunction (No 21 CFR compliance) |
| Manual intervention for aseptic filtration |
| Manual intervention for connection of vessels in same area/different area |

this all the parameters like reaction conditions, formulation components, and process parameters need to be fine-tuned to achieve desired product characteristics [87]. During liposome manufacturing depending on the technique of manufacturing several parameters need to be considered for process optimization. In the industry, the most widely adopted technique is conventional thin-film hydration and ethanol injection technique. In the conventional thin-film hydration technique CPPs are evaporation pressure, hydration time, hydration temperature, and sonication time. Whereas in ethanol injection technique the CPP includes rate of injection, temperature of aqueous phase, ratio of volume of solvent to aqueous phase, agitation rate, etc. [88].

- *Equipment Selection and Modification:* During R&D phase of product development careful selection of equipment needs to be done. It is essential that the equipment used for a particular manufacturer has different capacity of same equipment so that the transition to industrial scale is without any issues. Furthermore, any modification to existing equipment or investing in new equipment capable of handling larger volumes and higher throughput needs to meet the necessary qualification certificates. Ultimately the equipment used in industrial scale should be able to handle the necessary volumes required for production [89].
- *Manufacturing Vessels and Material Compatibility:* Material of construction for the pharmaceutical vessel is very critical aspect for design of the manufacturing vessels to ensure the safety and quality of the pharmaceutical product. A wide variety of materials are used for manufacturing of theses equipments.

If MOC of vessel is not compatible with product, then there are chances of contamination. So, the selection of MOC for vessel is very important both for construction of processing equipments and its associated accessories for manufacturing and storage of bulk formulation [90]. Various factors are considered for selection of MOC for pharmaceutical vessel such as extractable and leachable from MOC, pH resistance, compatibility with process solvents, etc. [91].

In general, product contact parts of pharmaceutical manufacturing vessels are made with various grades of stainless steel, majorly SS316L is used, whereas for product non-contact surfaces SS304 is used. Other grades of stainless steel are also used, such as SS316Ti and SS904L [92]. For acidic products, various grades of Hastelloy are also used like C-22, etc. For the products, which are not compatible with the metal, other materials of construction are also used like glass or PTFE. Vessel can be made entirely of glass or metallic vessels/components are lined with glass or PTFE. Different glass grades can be used for manufacturing. Three types of glass grades are available that are used in pharmaceutical industry: Type I, Type II, and Type III [93]. Glass type having the highest hydrolytic resistance and inert nature is Type I glass. This grade is used when the non-reactive nature is required or the product may be incompatible with SS grade vessels. Often the finished product is filled in this grade of glass.

During selection of MOC for pharmaceutical vessel, compatibility with cleaning agent is also considered, as to remove product residues, cleaning agents with higher or lower pH are also used.

- *Vessel Shape:* Pharmaceutical manufacturing vessel geometry typically depends on the specific product manufacturing and the required outcomes. Generally, vessels are of cylindrical shapes with torispherical dish ends or conical vessels. Cylindrical vessels have the advantages of simple structure, easy manufacturing, and convenient installation of accessories inside. Manufacturing vessels are designed in such shape that there is no retention volume left inside the vessel, when whole bulk product is required to be transferred from the respective vessel.

Cylindrical vessels are popular in pharmaceutical manufacturing for their simple design and efficiency. Cylindrical vessels offer uniform mixing and easy cleaning, making them suitable for a wide range of processes such as blending, storage, and reaction. Straight walls of the cylindrical vessels facilitate heat transfer and minimize dead zones, where materials can accumulate. Overall, cylindrical vessels are versatile and widely used in pharmaceutical production [94].

Conical vessels are commonly used in pharmaceutical manufacturing for processes that involve separation or sedimentation, such as centrifugation or crystallization. Conical shape of vessels is used, where phase volumes are very small and stirring has to be done with very low volume [95]. The tapering shape of the vessel facilitates the settling of solids, allowing for efficient separation from liquids. Additionally, conical vessels are often easier to drain completely compared to cylindrical vessels, reducing product loss and facilitating cleaning.

- *Agitator:* Agitators play a critical role in pharmaceutical vessel operations by promoting mixing and ensuring uniform distribution of materials. Agitators can be mounted on the bottom or top of the vessel, or sometimes both, depending on the application. Agitator helps in enhancing the mass transfer, heat transfer, and reaction kinetics, ultimately improving process efficiency and product quality [96]. Generally top-mounted agitators are installed from the center of the top dish of the vessel. Impellers of the agitators are built-in various designs, including propellers, turbines, and paddles, each suited to different mixing requirements. Bottom-mounted agitators are generally coupled magnetically and installed from the bottom dish of the vessel. Bottom-mounted agitators are installed off-center as generally in the center, discharge valve is installed [97].

Depending upon the requirements of mixing efficiency, speed of the agitators is defined. Speed of the agitators is controlled to set point by installation of variable frequency drive.

Depending upon the speed and working pressure requirements in the manufacturing vessel, top-mounted agitators are installed either with single mechanical seal or with double mechanical seal [98]. Double mechanical seals are either lubricated with

gas or product compatible liquid. If the product is sterile inside the vessels, mechanical seals also need to be sterilized.

- *Capacity Planning:* Careful assessment and planning of batch size to scaled and necessary requirements need to be considered. Furthermore, considering the market needs it is essential to consider factors like anticipated demand, production timelines, and resource availability at the manufacturing site [91].
- *Process Validation*: Validating the scaled-up manufacturing process is necessary to demonstrate its reliability, consistency, and reproducibility. This involves conducting comprehensive validation studies to confirm that the process consistently produces products that meet predetermined specifications.
- *Risk Management:* Careful risk assessment needs to be done to identify CPP, CMAs that can affect the CQA. Timely identification and mitigation of potential risks related to scale-up shall ultimately minimize the chances of production failures or quality issues. Concerned departments need to do risk assessments, implement contingency plans, and continuously monitor all the manufacturing processes for deviations or anomalies [99].
- *Technology Transfer:* If scaling up involves transferring technology from the laboratory to a larger production facility or contract manufacturing organization (CMO), effective technology transfer processes must be established to ensure seamless knowledge transfer and maintain product integrity.
- *Continuous Improvement:* Implementing a culture of continuous improvement is essential for optimizing production processes, reducing costs, and enhancing product quality over time. This involves soliciting feedback, analyzing performance data, and implementing corrective actions to drive ongoing improvements. By developing robust scale-up capabilities, nanomedicine manufacturers can effectively transition from small-scale research and development to large-scale commercial production, thereby meeting market demand and delivering innovative healthcare solutions to patients.

*3.1.1.1.4.5 Ethanol Injection Technique: Scale-Up* Ethanol injection technique has been used since 1990 for the preparation of liposomes. This method is preferred in industrial scale as it is easy to implement and scale, the method is reproducible, and it does not cause lipid degradation and oxidative alterations [100]. Using this technique, it is feasible to form SUVs even during large-scale productions. When the ethanol phase containing phospholipids are added to the aqueous phase it leads to precipitations of lipid molecules in the form of planar fragments. Energy needs to be provided for the planar bilayer fragments to spontaneously enclose into bilayered vesicles and hence this technique is often accompanied with stirring and/or ultrasonication. This causes the lipid bilayer to decrease the exposure of their hydrophobic regions to aqueous phase and induce curvature leading to formation of quasi-spherical structure [21]. Several factors like lipid composition and concentration, injection velocity, temperature of both the phases, stirring rate, sonication amplitude and time, etc., need to be considered during manufacturing of liposomes by ethanol injection technique [101–103].

Charcosset et al. have explained the scaling up of ethanol injection technique for preparation of batches 60 mL to 3 L. Scale-up was done using syringe as injector at small scale to use of pumps for injectors using membrane for larger scales. Post injection and formation vesicles the ethanol was removed under reduced pressure. Following is the brief regarding different scale techniques used for manufacturing using ethanol injection [104].

i. Syringe Injectors (60 mL batch size): The organic phase with phospholipids was injected into aqueous phase using a syringe at a flow rate of 1.5/min. The system was stirred using magnetic stirrer and addition of organic phase to it resulted in formation of milky solution for defined time interval. Then, the ethanol was removed using rotary evaporator under reduced pressure.
ii. Membrane Injectors (600 mL batch size): In this technique two positive displacement pumps were reduced. One pump circulated the aqueous phase from inlet of tubular membrane to the outlet and the second pump assisted in circulation of organic phase containing phospholipids. The second pump consisted of membrane device with pores through which the organic phase was brought in contact with aqueous phase. The flow rate of both the phases was set such that they passed at the same time. The prepared liposome was kept under stirring post which the organic phase was removed under reduced pressure. In this technique, after manufacturing of liposomes it is essential that the membranes are regenerated using proper methods. The outer and inner sides of the membrane need to be washed with suitable volume of aqueous or organic phase and ensure all the lipids, excipients, and API are removed and membrane permeability is observed to be around 90% of its initial value.
iii. Pilot Plant with Tube Injectors: In this technique aqueous phase and organic phase containing phospholipids are kept in separate double-jacketed reactor vessels and maintained at temperature above the phase transition of lipids. The reactor vessels had impellers required for continuous agitation of the phases. The organic phase was added to the aqueous phase using tube having a defined internal diameter. Post addition of lipid organic phase to aqueous phase, liposomes were formed. The remaining organic solvent was then removed under reduced pressure.
iv. Pilot Plant with Membrane Injection: In this technique the aqueous was kept in a double-jacketed reactor vessel under continuous agitation. A peristaltic pump was used to circulate aqueous phase inside a tubular membrane. The second phase was organic phase which was injected using positive displacement pumps through membrane pores into circulating aqueous phase. The formed liposomes were collected in another double-jacketed vessel. The flow rate was set such that both phases passed at the same time. The liposomes collected were then subjected to removal of organic phase under reduced pressure.

Liposome manufacturing is a complex process requiring critical considerations to process involved. The schematic representation of liposome manufacturing flow is given in Figure 3.5.

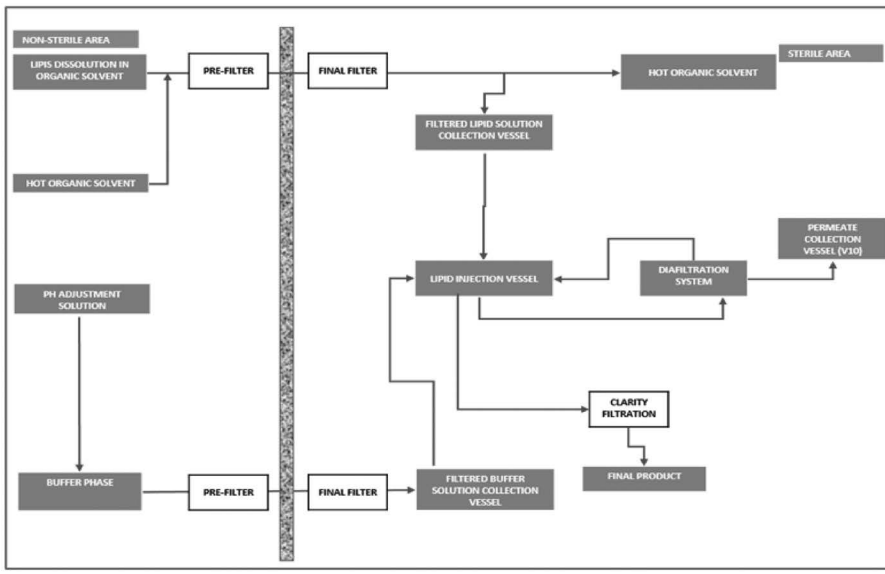


**FIGURE 3.5** Process flow for liposome manufacturing.

## 3.2 SCALE-UP PREDICTION USING MATHEMATICAL EQUATIONS

As discussed above the manufacturing of liposomes using ethanol injection technique requires large cylindrical tanks. The liquid present in the tanks requires efficient mixing using impellers or other mixing aids. It is essential that criteria like energy dissipation rate, vessel size, impeller tip speed, viscosity and density of liquid phase, Reynolds number, etc., need to be considered [105, 106]. Scale-up and process efficiency can be predicted using mathematical equations in such conditions.

The power (P) required for a process to run efficiently depends on following factors which are mixing rate (N) required, diameter of the impeller (Di) required for the mixing in the vessel of volume (V), density of the liquid (ρ) in kg/m$^3$. This can be expressed using Equation (3.1) given below where Np is power number.

$$P = NpN^3Di^5\rho \tag{3.1}$$

Np, which is the power number, is a proportionality factor that depends on impeller design and other factors and remains constant across different scales when same type of impeller is used. In the case of turbulent flow and Np constant, the equation changes to

$$P \alpha N^3Di^5 \tag{3.2}$$

Furthermore, the volume of cylindrical tank used for mixing of solution is given by

$$V = \left[\pi\left(D_T^{\,2}\right)\right] \times H/4 \tag{3.3}$$

where $D_T$ is diameter of a cylindrical tank and H is the height of a cylindrical tank.

Thus during scale-up, assuming the ratio of power input per unit volume is constant in two different capacity vessels and that geometric similarity is applicable ($D_T$ $\alpha$ Di and H $\alpha$ Di) and keeping Di constant it gives us:

$$\left(P/V\right)_1 = \left(P/V\right)_2 \tag{3.4}$$

Since

$$P/V\alpha\left(N^3Di^5\right)/V,$$

we have

$$\left(N^3/V\right)_1 = \left(N^3/V\right)_2 \tag{3.5}$$

Thus, with this equation we can establish the relationship between mixing rate and the vessel volume while scaling up.

## 3.3 WASTE MANAGEMENT SYSTEM

Waste management systems are critical in nanomedicine manufacturing to ensure proper handling, disposal, and recycling of waste materials generated during production processes. Here are key components of waste management systems in this context:

- *Hazardous Waste Identification:* During the development of nanomedicines and its manufacturing, the processes may generate different types of hazardous waste, may have chemical reagents, solvents, contaminated equipment, and disposable materials. Identification and categorization of these wastes on the basis of their hazardous nature need to be done considering the regulatory requirements [107].
- *Segregation and Storage*: All the hazardous waste should be segregated from non-hazardous waste at the point of generation to prevent cross-contamination and facilitate proper disposal. Furthermore, all the waste based on their nature should be clearly labeled, well-ventilated, and equipped with appropriate containment measures to minimize risks to personnel and the environment [108].
- *Chemical and Biological Waste Treatment:* Hazardous chemical waste and biological waste should be treated or neutralized using appropriate methods before disposal. This may involve techniques such as chemical neutralization, autoclaving, or incineration to render the waste non-hazardous and safe for disposal [109].
- *Recycling and Reuse:* Efforts should be made to use green technology and minimize solvent loss. Solvents removed during rotary evaporation can be collected and reused. Similarly, wherever feasible, efforts should be made to recycle or reuse waste materials to minimize environmental impact and

conserve resources. Closed-loop systems can be implemented to recover and reuse by-products generated during the manufacturing process [110].

- *Disposal Practices:* Hazardous waste that cannot be recycled or reused must be disposed of in accordance with applicable regulations and guidelines. This may involve contracting with licensed waste disposal companies to transport and dispose of waste at authorized treatment facilities, such as chemical incinerators or hazardous waste landfills [111, 112].

## 3.4 CONCLUSION

Development and manufacturing of formulations like liposomes are complex in nature and require extensive scientific and process understanding. Scaling up of formulation after development at lab scale to manufacturing at lab scale needs several considerations. Various infrastructural requirements for such manufacturing need to be considered in detail. Equipments and instruments used in manufacturing to be validated and should confirm with the regulatory guidelines outlined. It is essential that scale-up and infrastructural requirements are taken into consideration during developmental stage. Proper risk assessment needs to be done to understand critical process and material parameters and consider the same during large-scale manufacturing. Furthermore, continuous compliance monitoring needs to be done to ensure the process, systems, and necessary facilities are validated. The pharmaceutical waste generated requires regular monitoring and there is a need for auditing waste management practices to ensure compliance with regulatory requirements and environmental standards. This includes maintaining accurate records of waste generation, handling, and disposal activities, as well as conducting periodic inspections and assessments of waste management facilities.

Any regulatory queries and issues raised along with ethical considerations associated with the development and commercialization of nanomedicine products need to be addressed by the researchers. This includes navigating regulatory pathways for product approval, conducting preclinical and clinical studies to demonstrate safety and efficacy, and addressing ethical concerns related to patient privacy, informed consent, and equitable access to healthcare technologies. Overall, nanomedicine R&D plays a crucial role in advancing the field of medicine by harnessing the unique properties of nanotechnology to develop innovative solutions for diagnosis, treatment, and prevention of diseases, ultimately improving patient outcomes and quality of life.

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# 4 Synthesis of Nanostructures
## *Bottom-Up and Top-Down Approaches*

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### 4.1 INTRODUCTION

Nanotechnology is playing a vital role in the manufacturing of nanostructures (NSs) for diverse applications in medicines, cosmetics, bio-sensors, nanofiber production, and more. These structures with reduced size have a significant effect on their physical, chemical, thermal, and mechanical properties. Additionally, the shape of nanoparticles (NPs) also influences their physicochemical properties. For instance, the radiation and excitation wavelength of metal NPs can vary with respect to changes in the size of that NP. This distinctive attribute of metal NPs can be linked to an optical phenomenon referred to as localized surface plasmon resonance (LSPR) [1].

The NSs are of three different types including incidental, natural, and engineered [2]. Incidental NSs are industrial by-products while natural NSs are obtained through the natural process. In contrast, the manufacturing of engineered NSs entails specific techniques due to their intricate structure [3].

Two chief techniques such as bottom-up and top-down are employed in the manufacturing of different types of NSs. In the bottom-up approach, NSs are built up from the bottom through atom by atom or molecule by molecule. This atom or molecule deposition over each other causes self-assembling into NSs. The synthesis of any product using chemical reaction is the best example of a bottom-up approach. The top-down approach involves cutting bulk material into nanosized particles [4]. Both bottom-up and top-down approaches are presented in Figure 4.1. Among these approaches, bottom-up can be economical however; achieving control over it is somewhat difficult. The top-down approach is suitable for large-scale manufacturing of NSs and chemical purification of the product is not required with this technique. On the other hand, the bottom-up approach has some limitations for large-scale manufacturing and the purification of product is required. The selection of an apt

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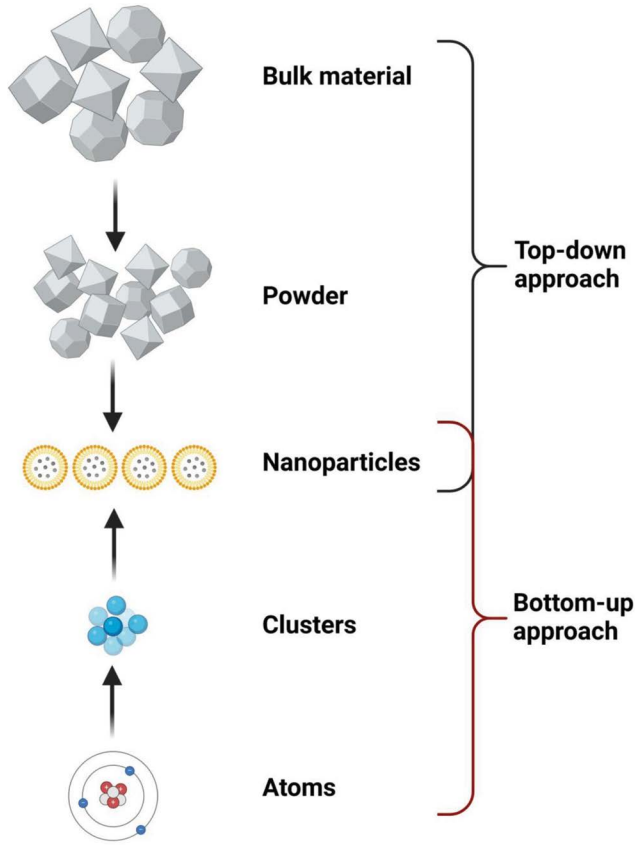


**FIGURE 4.1** Representation of top-down and bottom-up approaches of NSs synthesis. The bottom-up technique involves making NSs from smaller particles like atoms or molecules *via* chemical or physical processes. On the other hand, the top-down process begins with bulk materials and uses techniques like lithography or milling to break them down into NSs.

technique in the manufacturing of NSs is important to appropriate particle size and shape and thereby related physicochemical attributes [5]. This chapter covers diverse bottom-up techniques used for the synthesis of NSs. Moreover, various top-down techniques used for the fabrication of NSs are also discussed.

## 4.2 SYNTHESIS APPROACHES FOR NSs

The NSs or nanomaterials can be manufactured using two main approaches including bottom-up and top-down. In bottom-up approach nanomaterials are synthesized by building upon individual atoms or molecules. On the other hand, in order to create nanoscale structures, the top-down method entails lateral designing of bulk materials using either additive or subtractive techniques. The bottom-up approach involves liquid and gas-phase synthesis of nanomaterials [5]. Notably, this technique

is capable to manufacture NSs of the size ranging between 2 and 10 nm [6]. In the top-down approaches, mechanical, chemical, or thermal ways are employed for size reduction of bulk material during NSs synthesis. Despite of major role of top-down approaches in synthesis of NSs, it has several downsides like need of high surface finished materials, lack of perfections in manufactured materials, elevated etching times, and huge cost [7]. Both approaches are explained in below sections.

### 4.2.1 Bottom-up Approaches for the Synthesis of NSs

Nanostructured materials are used in a variety of nanotechnology fields, including nanoelectronics, optoelectronics, bioelectronics, nanochemistry, sensing, nanofluidics, point-of-care diagnostics, nanomachines, therapeutics, and advanced energy storages. Diagnostic and therapeutic applications are rapidly evolving in response to the growing demands of real-time industries such as medical and health care. The bottom-up approach employs and combines sub- or nanoscale components (atoms or molecules) to create NSs with novel or different functions (Figure 4.2). This strategy, which allows for more controlled system design, can be seen as the logical progression of supramolecular chemistry, which focuses on intermolecular links and investigates the structures and functions of the entities created by the association of two or more chemical species [8].

#### 4.2.1.1 Microwave-Assisted Method

Microwave-assisted synthesis is a promising technique for creating NSs quickly and efficiently. Microwaves, a type of electromagnetic energy, are used in this method to generate heat *via* two main processes such as polarization and conduction. This

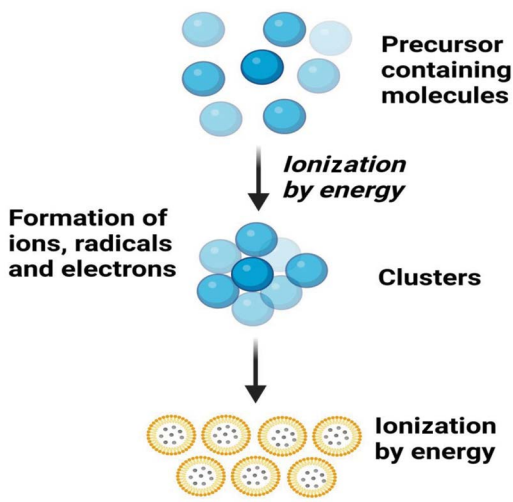


**FIGURE 4.2** Bottom-up approach for NSs synthesis. The bottom-up approach for NSs synthesis includes constructing NSs from atomic or molecular precursors through techniques like chemical vapor deposition, precipitation, etc.

heating approach is frequently employed in the synthesis of many materials, including ceramics, polymers, and metals, leading to higher reaction rates, shorter processing times, and high product yields.

Microwaves improve crystal nucleation and growth during NSs synthesis, providing benefits such as precise and controlled heating. The technique frequently produces materials with unique morphologies, such as sharp edges and well-defined surface features, which are critical for applications that require high surface activity, such as catalysis or surface-enhanced Raman scattering (SERS). Furthermore, polar molecules or solvents that efficiently absorb microwaves are employed to speed up the reaction process. Microwave-assisted synthesis is an eco-friendly, scalable, and cost-effective method for creating nanomaterials with desired properties. Silver and gold NPs produced through microwave irradiation have been used in drug delivery systems. These NPs enhance drug delivery and release profiles, increasing treatment efficacy by targeting specific tissues or cells with minimal side effects [9].

#### *4.2.1.1.1 Challenges of Microwave-Assisted Synthesis*

The ranges of challenges linked with microwave-assisted synthesis include non-uniform heating, limited applicability, control over particle size and morphology, and high equipment cost. Obtaining uniform heating throughout the material can be difficult, particularly in larger-scale processes. Microwaves primarily interact with polar molecules, causing uneven heating and potentially affecting the quality and consistency of synthesized nanomaterials. Moreover, certain materials, particularly non-polar substances such as metals in bulk, are poor microwave absorbers, limiting the effectiveness of microwave-assisted synthesis on these materials. Microwave synthesis frequently produces rapid growth and distinct morphologies, fine control over particle size, distribution, and shape can be difficult. Rapid heating can sometimes result in irregular or uncontrolled growth when compared to slower methods. Further, specialized microwave reactors are more expensive than conventional heating systems, which may limit scalability and widespread industrial adoption.

#### *4.2.1.1.2 Applications of Microwave-Assisted Synthesis*

This approach is used in NPs synthesis for catalysis wherein this technique allows for the rapid production of catalysts with large surface areas and active sites, increasing their efficiency in chemical reactions and environmental applications such as pollutant degradation and fuel cell development. Additionally, microwave-assisted synthesis has important applications in SERS. Microwave methods are used to create NS metal surfaces (e.g., silver and gold NPs) for SERS, which provide improved molecular detection due to their sharp-edged morphologies and are useful in biosensing and chemical analysis. Moreover, this technique has also demonstrated various pharmaceutical applications in which it is used to create nanomaterials for drug delivery systems, allowing for precise control over NP size and shape, which is critical for increasing drug bioavailability and therapeutic efficiency. Further, microwave synthesis is used in materials science to prepare ceramics and polymers, as it allows for faster synthesis and higher phase purity [10–12].

#### 4.2.1.2 Hydrothermal Method

The hydrothermal method is a popular way to create NSs because it allows precise control over particle size, shape, and crystallinity. This process involves crystallizing materials from aqueous solutions at high temperatures and pressures in a sealed autoclave. Under these conditions, water acts as both a solvent and a reaction medium, increasing reactant solubility and accelerating reaction kinetics. The controlled high-temperature and pressure environment promotes the development of various NSs, such as nanorods, nanowires, and NPs. Temperature, pressure, reaction time, and pH are all important parameters in the synthesis process. Temperature controls particle size and crystallinity, whereas pressure influences reactant solubility. Reaction time allows for fine-tuning of NSs, with longer durations typically producing larger particles. The pH of the solution is critical in determining the chemical phases and morphologies of the final materials.

The hydrothermal method's primary advantage is its ability to produce high-purity materials with well-controlled morphologies at relatively low temperatures. It is also environmental friendly, frequently replacing the need for hazardous organic solvents. However, challenges include the high-energy requirements to maintain high-pressure conditions and the difficulty in scaling up the process for industrial production. This method is especially useful for creating oxide NSs, including $TiO_2$ for photocatalysis, ZnO for sensors and optoelectronics, and hydroxyapatite for biomedical applications. Because of its flexibility and adaptability, the hydrothermal method is widely used in producing functional nanomaterials for applications in energy, environmental remediation, and health care [5, 8, 13, 14].

##### *4.2.1.2.1 Challenges of Hydrothermal Synthesis Method*

Prolonged reaction time is a key challenge of this method. Hydrothermal synthesis frequently requires long reaction times, ranging from several hours to days, especially for complex NSs. These lengthy processes impede scalability and efficiency, particularly in industrial-scale production, resulting in higher costs and energy consumption, reducing its suitability for high-throughput manufacturing. High-energy consumption is the second important challenge because this technique requires sustained high temperatures (100–250°C) and pressures (up to several MPa) over long periods, making it energy-intensive. This high-energy demand can be a barrier for cost-sensitive industries, particularly large-scale production of nanomaterials for commercial use. Although the hydrothermal method can control NSs morphology and size, precise control requires careful optimization of multiple parameters such as temperature, pressure, pH, and surfactants. Without proper regulation, inconsistent particle size and shape may occur, reducing the performance of nanomaterials in applications requiring uniformity such as catalysis, drug delivery, and electronics. Scalability and reproducibility also serve as important challenges. Maintaining uniform temperature and pressure across larger volumes is difficult when scaling hydrothermal synthesis from the lab to the industrial scale. Ensuring batch-to-batch consistency in nanomaterial properties is difficult because variations during scale-up can result in defects or lower-quality materials, limiting the method's suitability for mass production. Surfactants and templates are frequently used to guide the

formation of specific NSs. However, removing these organic additives after synthesis without affecting the NSs frequently necessitates additional purification steps. Residual organic compounds can have a negative impact on final material properties, especially in applications that require high purity, such as catalysis or biomedical devices. Further, limited material scope is another downside of hydrothermal technique. The hydrothermal method is excellent for synthesizing oxides and certain metal compounds. However, it is less suitable for non-oxide ceramics or metals that require different synthesis conditions. This limitation restricts the range of materials that can be synthesized using this method, limiting its broader application [15].

#### *4.2.1.2.2 Applications of Hydrothermal Synthesis*

This technique has a wide range of applications. Hydrothermally synthesized nanostructured materials like $TiO_2$ and ZnO are extensively used in photocatalytic applications, such as water splitting, environmental purification, and dye degradation. These NSs have high surface areas, controlled porosity, and tailored electronic properties, which improves their photocatalytic performance. It is also used to produce nanomaterials for energy storage devices like lithium-ion battery electrodes. Nanostructured materials, such as manganese oxides and cobalt oxides, improve battery performance by increasing surface area, charge/discharge rates, and stability, resulting in more efficient energy storage. Importantly, hydrothermally synthesized metal oxide NSs like ZnO and $SnO_2$ are widely used in gas sensing applications. NSs with high surface-to-volume ratios improve gas detection sensitivity and selectivity, including $NO_2$, $CO_2$, and $NH_3$. Their faster sensor response time and lower detection limits make them ideal for gas detection. Moreover, hydrothermal synthesis produces biocompatible nanomaterials such as hydroxyapatite for bone tissue engineering and silver NPs for antimicrobial coatings. These materials have excellent mechanical properties, bioactivity, and controlled drug release profiles, making them ideal for biomedical implants and drug delivery systems. Further, light-emitting diodes (LEDs) and photodetectors make use of hydrothermally synthesized nanomaterials such as quantum dots and metal oxides. Their enhanced optical properties, such as higher emission efficiency and tunable wavelengths, result in improved device performance in these applications [16–18].

### 4.2.1.3 Polyol Synthesis Method

The production of NSs utilizing process variables such as high boiling points and multivalent alcohols are referred to as the polyol's technique of NSs synthesis. This method is commonly used to fabricate metallic and metal oxide NSs. Different NSs can be fabricated using polyols such as NPs, nanorods, and nanowires. The fundamentals of synthesis rely on the use of polyols like ethylene glycol (EG), diethylene glycol, and glycerol [19]. Using polyols as solvent and as a reducing agent helps synthesize NSs with their desired morphology and composition. Polyols possess various unique characteristics that render them superior candidates for NSs creation, such as their solubility, which closely resembles that of water for simple metal salts and their predecessors, wide variety of boiling points (maximum of 320°C), the property of reduction allows for the rapid development of metal NSs, the broad spectrum of

approval based on authority guidelines, ranging from low-molecular-weight EG to high-molecular-weight polyethylene glycols (PEGs) [19, 20]. In this method, chemical reduction and decomposition of metallic salts and organometallic complexes are commonly conducted using various reducing agents such as hydrogen, carbon monoxide, and hydride salts. Polyol synthesis commonly uses metal precursors like metal salts, such as chlorides, nitrates, and acetates, which play an essential role in dissolving polyols [21]. The polyols method is a versatile, reliable, and scalable approach to the production of NSs, particularly metal NPs and oxides. The approach provides substantial control over nanomaterials' size, shape, and morphology making it useful for various industrial and scientific purposes [22, 23].

At a specific temperature, the metal salts precursors are dissolved in polyols. Here, these polyols act as solvents. In the next step, nucleation and growth take place with increasing temperature. The reduction of metal ions to its metal atom happens at a critical concentration where nucleation occurs, followed by the growth of nanocrystals. The controlled growth stage is crucial in defining the morphology of the structure, influenced largely by factors like temperature, concentration, and the stabilizing effects of the capping agent. The capping agent, depending on its concentration, selectively binds to different crystal faces. The next step is isolation and purification, where, after a desired morphological character, NSs are isolated from bulk, with external forces like centrifugation [22]. Excess amounts of reagents are removed by washing process to get pure NSs.

#### 4.2.1.4 Microemulsion Technique

The fabrication of NSs by microemulsions (MEs) involves encapsulating a substance within a nanoscale wall material, enabling control over the size and surface area of the resultant particle. The ME is thermodynamically stable and isotropic dispersions of two immiscible liquids [24]. A surfactant stabilizes microdomains in conjunction with a co-surfactant. With an average droplet diameter ranging from 10 to 140 nm, these self-aggregated colloidal systems can be categorized as bi-continuous ME, which consists of water and oil nanochannels separated by a flexible surfactant monolayer, oil-in-water (O/W) ME, where oil is dispersed in water, and water-in-oil (W/O) ME, where the water is dispersed homogeneously in an organic medium [25–27].

In this technique, the first step, involves melting the solid lipid and combining it with the liquid lipid. The drug is then dissolved in this lipid mixture. In a separate phase, the surfactant and co-surfactant are added to the aqueous solution. Both phases are maintained at a temperature higher than the melting point of the solid lipid and then mixed together to create a hot ME. Next, the hot ME is diluted with excess cold water while being stirred. This dilution process causes the ME to break apart, transforming it into ultrafine nanoemulsions. Over time, the internal lipid phase of these nanoemulsions gradually recrystallizes, resulting in the formation of nanostructured lipid particles [26]. The various benefits of this technique include regulation of particle size enables meticulous control over particle size and dispersion by managing droplet size within the ME. In addition, high thermodynamic stability, versatility in the synthesis of nanomaterials, such as oxides and metals, synthesis at modest temperature and pressure, and ease of industrial scalability are other important benefits [27].

#### 4.2.1.5 Solvo Thermal Method

Solvo thermal production is a versatile method for developing a wide range of NPs with tiny dispersion. The solvo thermal method, like the technique known as hydrothermal, can be applied to solvents other than water. NPs and nanorods with and without surfactants have been produced using the solvo thermal method, which offers greater control over shape, size, and crystallinity distributions compared to the hydrothermal technique. The procedure entails chemical reactions in a solvent at elevated temperatures and pressures, often in a sealed autoclave [28, 29]. The specific parameters such as temperature, pressure, solvent type, and reaction time are often tuned based on the material system and the desired NP properties [30]. This technique offers various benefits such as NPs with high-purity and well-crystallized NPs with homogeneous shapes. Additionally, it allows for the synthesis of materials that are difficult to acquire using other procedures, especially at low temperatures. The utilization of closed systems (e.g., autoclaves) provides for high-pressure settings, allowing reactions that would not otherwise occur. Furthermore, solvo thermal synthesis is adaptable to a diverse spectrum of materials, including oxides, sulfides, and metals [28, 29].

#### 4.2.1.6 Sol-Gel Method

The sol-gel method is a wet chemical process to produce NSs, namely metal oxide NPs. The technique involves dissolving a molecular precursor (usually metal alkoxide) in water or alcohol, then heating and stirring it to form a gel by hydrolysis/alcoholysis. Because the gel produced during the hydrolysis/alcoholysis process is wet or damp, it needs to be dried using the right techniques based on the application and desired qualities of the gel [31, 32]. High purity and homogeneity, low processing temperature, ability to produce various NSs, control over particle size and morphology, large scale and porous material production, cost-effective tailored surface properties, and environment-friendly approach are key benefits of sol-gel method [33, 34].

#### 4.2.1.7 Chemical Vapor Synthesis

Chemical vapor synthesis (CVS) can be done *via* various strategies like chemical vapor precipitation, chemical vapor decomposition (CVD), and chemical vapor reaction procedures. This approach uses chemical processes at extremely high temperatures to deposit solid coatings from the vapors phase. The quality of material deposition is heavily influenced by precursor content, reaction rate, and temperature. This approach is widely utilized in the semiconductor industry to create clean and thin films with good performance. Under specific conditions, the CVD process produces ultrafine particles, resulting in thin films. CVD processes often expose the substrate to volatile precursors [35]. The precursors exist in three states mainly solid, gas, and liquid, which are synthesized and created as vapors in the reactor under particle nucleation conditions. CVD processes often expose the substrate to volatile precursors. CVD is commonly used for developing thin nanostructured materials layers of crystalline inorganic materials such as ZnS, CuSe, InS, and CdS [23, 36].

In the CVD method of NPs synthesis reactant gasses are first introduced in the reactor. Reactant gases can either undergo homogeneous gas phase reactions to produce intermediate reactants and by-products or diffuse directly onto the substrate

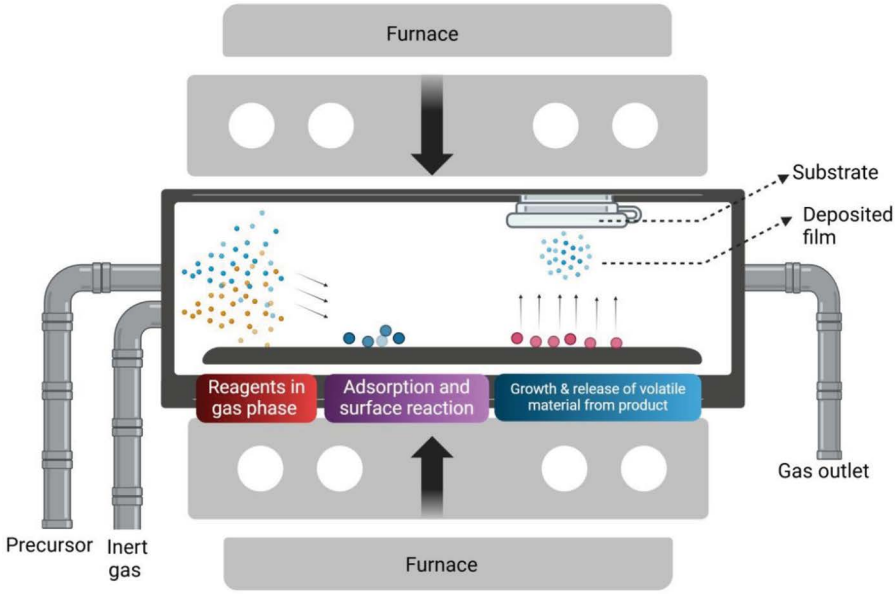


**FIGURE 4.3** The CVD technique for synthesis of NS. In CVD for NSs synthesis, gaseous precursors are added into a heated reaction chamber where they react or breakdown to generate solid NSs.

*via* a boundary layer. In both cases, intermediate reactants and reactant gases adsorb and diffuse onto the heated substrate surface [37]. Evaporation transports developing organisms to a substrate for assembly and growth *via* a carrier gas. Heterogeneous processes at the gas-solid interface produce thin films and by-products through coalescence, growth, and nucleation. Unwanted gaseous products and undissolved species are eliminated from the reaction zone's surface over time. Gas-phase reactions occur whenever the temperature is high enough, or additional energy is provided, such as plasma. Furthermore, a heterogeneous response is needed if the deposition reaction depends on substrate surface catalysis, as with some metal surface catalytic growth [23, 35]. The CVD techniques for NSs synthesis is depicted in Figure 4.3. The ranges of advantages of CVD method are the process of CVD has the finest degree of control, it gives high-purity NSs, easy to control process parameters allowing regulating crystal structure, surface morphology, and orientation of NSs, and excellent substrate uniformity and material adhesion allow for intricately formed coatings [36, 37].

#### 4.2.1.8 Precipitation Method

Precipitation is one of the oldest wet chemical methods used in the synthesis of nanomaterials. This is the most fundamental and widely applied method for synthesizing a variety of nanomaterials. In precipitation methods, the metal precursors are dissolved in a common solvent, like water, and an insoluble solid is produced by adding precipitating agents. The main advantage of precipitation processes is that they generate enormous volumes of particles. A homogeneous precipitation reaction is

usually performed to make uniform particles, separating the nucleation and developing nuclei [21, 38]. The most popular and appropriate technique for creating MNPs with precise sizes and magnetic characteristics is co-precipitation. Because of its simplicity of use and reduced requirement for potentially dangerous substances and processes, it finds widespread application in the biomedical field. This technique makes MNPs from aqueous salt solutions by adding a base at room temperature or above while in an inert atmosphere. Using a precipitation reaction to accomplish a constant composition of two or more cations in a homogeneous solution is known as the co-precipitation strategy [39]. This method can immediately manufacture homogeneous nanomaterials with small sizes and size distribution through various chemical processes in solution. To maintain the necessary pH, the solution is mixed directly or drop by drop with another solution containing dissolved precipitation agents, such as ammonia, sodium hydroxide, and many more. Among these are oxalates, carbonates, hydroxides, and chlorides, most widely used as precipitating agents. After precipitates are aged to yield larger particles, filtration or centrifugation is used to gather the particles. It takes a second wash with ethanol, distilled water, or other solvents to eliminate impurities and produce high-grade NPs. Annealing, sintering, or calcinations post-treatments produce NMs with the desired crystal structures and morphologies [40, 41]. This method has several challenges like impurity precipitation may happen in the form of a precipitate, and the stabilization of prepared nanomaterials is required; this step may involve the addition of chemical reagents which can generate potentially hazardous waste material, and post-processing optimizations necessary for a pure final product [38–40].

#### 4.2.1.9 Rapid Expansion of Supercritical Solutions

Supercritical fluid technology has significantly impacted particle formation applications. A supercritical fluid is a solvent above its critical temperature that stays in a single phase independent of pressure. Fluids used in materials processing are often near-critical temperatures due to their high density and solubility. Supercritical fluids have low and tunable densities that can be changed from gas to liquid with a simple pressure change at a constant temperature. They also exhibit unique solvation effects at densities near the critical density, often referred to as solute-solvent and solute-solute clustering [42]. The precipitation processes in supercritical solutions known as SAS (supercritical anti-solvent) and RESS (rapid expansion of supercritical solutions) are among the processing approaches that have been studied the most. The decrease in pressure, which can go through the expansion nozzle up to the speed of sound, propels the RESS process. The rapid expansion from supercritical pressure to ordinary pressure results in extremely high supersaturation and therefore, uniform nucleation of the solute creating narrow size distributions in the products. This is why solubilities in supercritical fluids can be up to a million times higher than those under ideal gas conditions. The procedure includes saturating SCF with the substrate and depressurizing the solution through a heated nozzle into a low-pressure chamber. This causes fast nucleation of the substrate. As the solution expands through a calibrated aperture, its density gradually decreases, causing the solute to precipitate into finely divided solid fibers or crystals [43]. Expansion under high pressure increases particle density and slows flow velocity, allowing for clustering and aggregation.

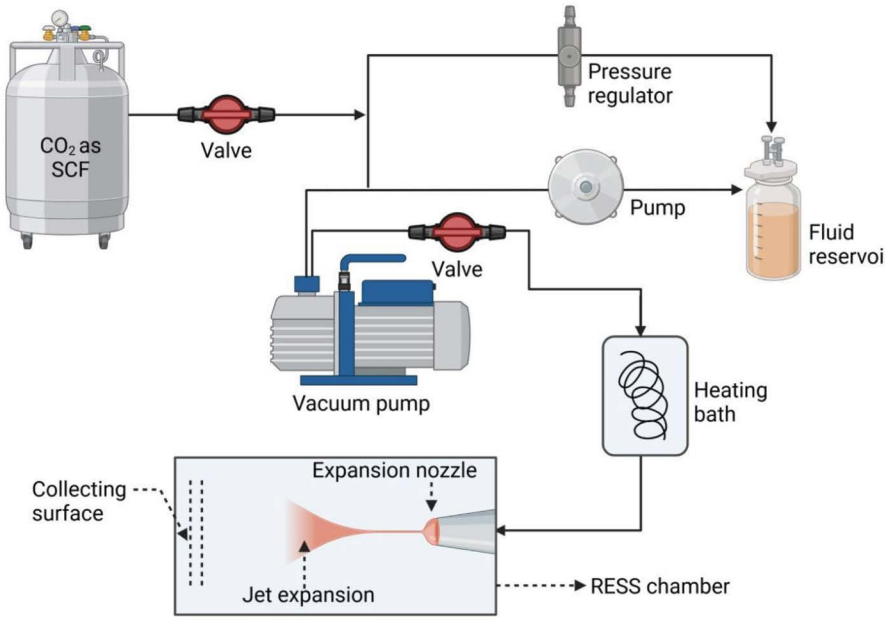


**FIGURE 4.4** The process of rapid expansion of supercritical fluids. This technique of NSs synthesis includes injecting a supercritical fluid into a chamber where it undergoes an abrupt decrease in pressure and temperature that causes nucleation and the formation of NSs.

High expansion pressure produces bigger particles. Expansion into low pressure leads to low density and rapid velocity, making flow time and density less conducive for cluster formation [44]. The process of rapid expansion of supercritical fluids is presented in Figure 4.4. This technique offers several advantages like precise mass transfer, rapid kinetics, a faster reaction rate, precise control over morphology and particle size, and solvent-free recovery [42–44]. While limited solvent choice and the high cost of the equipment are key challenges of this method [44].

### 4.2.2 Top-Down Approaches for Synthesis of NSs

The top-down approach of NS synthesis is simply the approach that decreases the size of the large structure toward the nanosized [23]. This approach involves a mechanical, chemical, or thermal strategy for size reduction of bulk material. It is the simplest way of nanosized fabrication. However, this technique is ineffective for obtaining properly sized and shaped NSs due to the manufacturing of irregularly shaped and smaller-sized particles [23]. The techniques used for top-down approaches are discussed below.

#### 4.2.2.1 Laser Ablation

Laser ablation (LA) removes the surface molecules by a high-intensity beam of electromagnetic radiation to create nanosized structures by vaporization and melt

ejection. It has various applications in polymers, metals, glasses, and ceramics. This technique involves laser cutting, laser beam milling, and high-precision drilling, which is the overall process of LA. Commonly used laser sources are near-infrared Nd:YAG, infrared, and UV/excimer lasers for size reduction.

This method is conducted in a vacuum, liquid, or gas medium, and NSs are fabricated. When the LA is performed in a vacuum medium, it is known as pulsed laser deposition. It offers the versatility to generate a nano- to micro-sized thin film along with controlled elemental composition. Whereas LA is performed in a liquid medium such as water, ethanol, or acetone, it is known as laser ablation in liquid (LAL). They are classified into two main types depending on the source of the laser source. If the laser source is continuous, then it is called continuous wave LAL (CLAL), and the pulsed used for laser ablation is known as pulsed LAL (PLAL). PLAL constantly emits light from a laser heat that heats up and induces the boiling of the liquid medium. That boiled liquid spreads the laser beam toward the targeted area. At the same time, the CLAL technique is unfeasible for large-scale production [45]. Many factors influence the laser characteristics including wavelength, fluency, transmission, repetition rate, pulse duration, light absorption efficiency, and chemical composition of liquid medium [46].

LA's advantages include the least energy loss and the creation of ligand-free noble NSs in various solvent mediums. However, it faces a few challenges like high ablation efficiency; large-scale NSs are not sensible, material adhesion, size control, and decreased ablation effectiveness with raised ablation duration [47]. The applications of the LA technique are finds across diverse fields, including medicine (drug delivery systems, imaging, etc.), catalysis (enhancing reaction rates and selectivity), electronics (fabrication of electronic components, sensors, photovoltaic devices, etc.), and environmental applications (degradation of pollutants and as antimicrobial agents) [45, 48, 49].

#### 4.2.2.2 Laser Pyrolysis

It is another technique for developing nanoscale material using a top-down approach. This method is commonly used for the synthesis of carbide (SiC, TiC, etc.) and ceramic ($SiO_2$, $TiO_2$, etc.) NPs by the interaction between laser photons, a gaseous phase and a sensitizer. Gaseous-phase precursors are attached to the chamber which is filled with carrier gas like argon and that linked with the laser beam. Moreover, a sensitizer works as an energy transfer component that is excited by absorption of $CO_2$ laser radiation and shifts the high energy to the reactant molecules through collision. The laser beam generates high power (2400 W) that increases the targeted area temperature which stimulates the process of nucleation and growth of NPs. These prepared NPs are collected by the catcher equipped. The laser pyrolysis process is characterized as a gaseous-phase process of nanomaterials synthesis, which does not mean that liquid mediums (chemical solutions) are not allowed. If the liquid medium is required, in this case, the reactants can be vaporized or converted into microscale droplets by sonication technique and induced into the reaction chamber. Therefore, this method is primarily used in the thermal energy induced *via* high-power lasers [50, 51]. At high thermal energy coaslescence is higher, leading to the formation of more spherical-shaped particles. By changing the process parameters,

the properties of nanomaterials can be changed. This method has tremendous advantages such as nanosized particles with high quality and narrow particle size distribution. Moreover, the process is continuous due to low side reactions resulting in the high purity of nanomaterials [52]. However, the major challenges of this technique are high initial cost and limited industrial point of view scalability [53]. Furthermore, the intense nature of the laser beam, which produces local temperatures of more than 10,000°C, creates a risk of recombination reactions to yield the major product and degradation of the product [54]. This technique is applied in many fields like chemistry, electronics, electrochemicals, medicine, and others. In chemistry, it is used as a catalyst for material production and analysis purposes. Meanwhile, in electronics, it is a component of electronic devices (solar cells, sensors, displays, superconductors, semiconductors). Furthermore, in electrochemicals, it is applicable in batteries, supercapacitors, and fuel cells. In the medical field, it is applicable in magnetic resonance, antitumor action, coating, or ingredients for plasma. Other fields include the environment, energy production, food production, aerospace, etc.

#### 4.2.2.3 Physical Vapor Deposition

Physical vapor deposition (PVD) is a vacuum deposition technique used to generate thin films and coatings. It is characterized by a process in which the material condenses first, vaporizes, and finally returns to the thin-film condensed phase. It includes sputtering and evaporation processes. The steps involved in PVD are sputtering/evaporation, supersaturation, and consolidation. In the sputtering/evaporation process, components are converted into the vapor phase. The vapor phase undergoes supersaturation in an inert atmosphere, which encourages the formation of metal NPs as the vapor condenses. The final step involves the thermal treatment of the material under an inert atmosphere, consolidating the NPs into a stable nanocomposite.

This process has numerous advantages, such as excellent durability, broad application, eco-friendliness, ease of maintenance, temperature resistance process abrasion, and impact resistance [55, 56]. However, there are a few challenges that PVD technology is facing, such as high cost and capital investment, the need for expert operators, difficulty in coating complex geometries or undercuts, and slow deposition rate [57]. The industrial application of PVD involves toolmakers, aerospace and automotive, jewelry and design, optics, and semiconductors. In toolmakers, it protects the material from corrosion, while in aerospace and automotive, it is used to enhance the wear resistance of metal portions and make it corrosion-resistant. Moreover, this technique is used for coating jewelry and design. Furthermore, optics creates a highly sophisticated mirror to coat the glasses for protection, reflection, or absorbing layer. Additionally, this technique is used in semiconductors to prepare microchips and thin-film photovoltaic cells using platinum, and tungsten sputter coated [58].

#### 4.2.2.4 High-Energy Ball Milling (HEBM)

HEBM is the simplest and most efficient way of constructing the NSs using a mechanical grinding approach to reduce the particle size up to the nanoscale. It is a versatile tool that works by mechanical energy in which the material is incorporated in a rotating chamber loaded with balls, and the balls impart kinetic energy to the

material, which results in significant changes in the structure of the incorporated material that makes nanoscale particles. Temperature and pressure are two parameters generated between the balls and the impact of balls and vessel walls, resulting in phase transformation at high temperatures. A fixed quantity of material fills the milling vial, and due to the friction and interaction between the vial and balls, the size of particles reduces to the nanoscale. This method significantly impacts the crystallite size, mechanical dislocation (reduction of particles), material surface modification, and chances of development of metastable phase. Various reactions can be carried out in this method, which are not performed at room temperature. These approaches are used for many purposes like compression, particle size growth, mixing two components, altering the particle structure, aggregation, etc. Several factors influence the milling process, including the size of the balls and vial, weight of balls, rotating speed and duration mill, total volume of powder material, etc. When the ball-to-powder mass ratio is higher, milling is impactful due to a higher collision rate. The milling balls and the vials moment decide the type of mill, such as planetary, horizontal, rotatory, attrition, and vibration mill.

HEBM has various benefits, such as large capacity and acceptance, low maintenance, reliability, safety, and simplicity, and it is also used for large-scale production. However, some challenges have to be faced using this technique, such as destruction, a disorder of crystal structure, induced flaws, inconvenience and hefty weights, high-energy requirements, noise, unsafe for heat-sensitive material, and easy contamination.

#### 4.2.2.5 Inert Gas Condensation

This technique is a type of top-down approach to nanosynthesis, having the capability and flexibility to manage the synthesis process. Simply, the components are evaporated under a controlled environment, and a cooling agent condenses the evaporated molecules to finalize the formation of nanosized particles. Primarily, three factors influence the nanosynthesis, namely the type of inert gas, the flow rate of forced gas, inert gas temperature, and pressure. The basic principle involved in the synthesis of nanoscale material is evaporation until the described size and morphology of the particle [19]. The insert gases such as He, Xe, or Ar are introduced to the chamber at low pressure and constant temperature. This point is where the nanosized particles are collected on the surface, which is cooled by the cooling agent. By using carbon-coated electron microscopy, the nanoscale particles are collected and segregated according to particle size [59]. These techniques have several benefits in the synthesis of inert metallic compounds, alloys, ceramic compounds, composites, and semiconductors. It provides flexibility to synthesize cluster size, desired size, and distribution using pressure and temperature parameters. Compared to the traditional method of nano synthesis, the inert gas condensation approach overcomes the obstacle of phase and kinetic equilibria by using diffusion distance, driving force, and uncontaminated surface, leading to surface cleanliness and nanoscale particles. Prominently metallic NPs such as manganese, nickel, iron and iron oxide, and gold/palladium NPs are synthesized using this technique [60, 61]. However, the major challenge of this technique is it requires precise condition temperature and gas pressure [62, 63].

#### 4.2.2.6 Homogenization Technique

Homogenization technique is primary and classified into two main techniques namely high-pressure valve homogenization and microfluidizers. These techniques are briefly described below.

##### *4.2.2.6.1 High-Pressure Valve Homogenizer (HPVH)*

It consists of a highly pressured plunger pump along with a relief valve known as a homogenizing valve. The plunger pump helps to provide high pressure (100–2000 bars), and the valve adjusts the driving force to support the homogenization process. In this technique, the medicated suspension is passed through the highly pressured homogenization gap, which results in a nanoscale composition. The principle behind the method is high shear force and collision of particles due to the narrow homogenization gap that promotes high velocity to the particles. According to Bernoulli's theory, an elevation in the fluid dynamic pressure, the static pressure decreases below the boiling point of the water. At the time of boiling, the cavity generates in the form of gas bubbles, and that force is sufficient for the size reduction of material from the micro to the nanoscale. Moreover, the collision helps with the nanosizing of the material by increasing its velocity. Two factors directly influence the HPVH, namely homogenization pressure and number of homogenization cycles. As there is an increase in the pressure of homogenization, the lower particle size is obtained and vice versa. This technique provides several benefits like easy preparation of nanosuspension, scale-up with a bit of beat variation in batch-to-batch production, narrow size distribution, suitability for the parenteral formulation, and both diluted as well as concentrated formulation can be prepared. However, a few disadvantages have been faced, like the prerequisite of micronized material with high-speed mixers performed suspension is required before injected into the homogenization process.

##### *4.2.2.6.2 Microfluidizer*

This technology is based on the frontal collision of the liquid medium under a high pressure (1700 bar) to generate nanosized particles. Same as HPVH, here, there is also collision, shearing force, and cavitation forces principles, but having fixed geometry and uniform size distribution makes a considerable difference between HPVH. The chambers of microfluidizers are commonly designed in a Z and Y shape for the nanosynthesis. Z-shaped chamber provides better interaction due to the numerous zigzag-shaped designs of the chamber. This leads to more particle collision and shearing force, resulting in the dispersing of particles and size reduction. This method is commonly used for W/O emulsion, size reduction, disaggregation, dispersion, and cell disruption. This technique provides more advantages such as industrial range scalability, eco-friendliness, and continuous process of NP fabrication. Therefore, it has the potential for the translation process of manufacturing [64, 65].

#### 4.2.2.7 Aerosol Flow Reactor Method

This technique represents the engineering of nanoscale particles. It is the simplest, continuous, and effective NP synthesis method with uniform size distribution. It uses biocompatible solvents for nanosized particle synthesis. The principle of the aerosol flow reactor method is very simple. The medication is dissolved in biocompatible solvents, which are volatile, and this uniform mixture is atomized with

highly pressurized gas carriers into nanosized droplets. These droplets are passed through a heated tubular laminar air flow, where the volatile solvent evaporates, and the supersaturated medication with a gas carrier forms nanosized particles. Here, temperature, solution concentration, atomizer, drug solubility, and drug nature can influence the size of the particles. The temperature in the laminar airflow is used for the evaporation of the solvent. If the rate of evaporation is higher, it may result in undesired structure formation (hollow) due to the rapid evaporation of the solvent. If the solute concentration in the solution is higher, there is a chance of large-sized particle formation; therefore, the concentration of the solution should be optimized. The atomizer atomizes the solution in nanosized droplets, resulting in NP formation. The drug should be freely soluble in the solvent and does not cause any interaction with the solvent, which may relate to well-desired NP formation.

This technique has several advantages such as NPs prepared using this technique can be used for modern drug delivery approaches, uniform size distribution, controlled morphology, and nanoscale particles. However, the major challenge of this technique is the difficulty in obtaining nanoscale particles of drugs that are insoluble in organic solvents [66, 67].

#### 4.2.2.8 Supercritical Anti-Solvent (SAS) Method

The SAS method is used for the nanoscaling of lipophilic cargo, which is not performed by rapid expansion from supercritical to aqueous solutions (RESS). This process used supercritical fluid as an anti-solvent for NP synthesis. The principle of SAS is dissolving drugs in the organic solvents and making a homogeneous solution that atomizes into an excess flow of supercritical carbon dioxide ($CO_2$). This $CO_2$ quickly diffuses into the homogeneous solution and vice versa. The mixture becomes supersaturated leading to the rapid nucleation of particles into a nanosize. It is a batch-to-batch or continuous process of NP development. This process can be influenced by the efficiency of the atomization of organic solvents in supercritical fluid.

This technique has several benefits including the potential to fabricate NPs incorporated with the lipophilic cargo, an eco-friendly process, and easy removal of organic phase from the process of manufacturing. However, broad particle size distribution and batch-to-batch variation are the chief disadvantages of this method.

#### 4.2.2.9 Supercritical Anti-Solvent Enhanced Mass Transfer (SAS-EM) Method

This method is used to generate nanosized particles along with the narrow size distribution by virtue of enhancing the mass transfer. In this technique, supercritical fluid is used as an anti-solvent. The dispersion nozzle is attached to the ultrasonic frequency-generating vibrating units for enhancing the mass transfer of nanodroplets. Supercritical fluid is introduced on the vibrating surface united for generating a thin liquid film. This oscillatory moment forms helps wavelets to improve amplitude till the wavelets break resulted in quick and rapid expansion that reduces the droplet size and precipitation of drug moiety into nanosized particles by 10-fold as compared to traditional SAS. The vibrating intensity can influence the particle size and morphology of the NPs. The main advantages of this technique are scalable technology, narrow size distribution, better morphology, and nanosized particle formation; no specialized nozzles are required and can be used for novel drug delivery systems [68–71].

## 4.3 FACTORS INFLUENCING SYNTHESIS OF NSs

The size, morphology, composition, physicochemical properties, and overall stability of the synthesized NSs are influenced by a variety of factors, rendering them appropriate for their applications. These factors can widely be divided into choice of starting materials, method of synthesis, reaction conditions, post-synthesis treatment, and environmental factors.

### 4.3.1 Starting Materials

#### 4.3.1.1 Type of Starting Materials

Depending on the type of precursor, different classes of NSs can be synthesized like deposition of carbon precursors leading to the synthesis of carbon-based NSs (e.g., fullerenes, carbon nanotubes), metal NSs (e.g., gold, silver, zinc, copper, iron NPs), ceramics NSs synthesized from inorganic precursors (e.g., aluminum oxide ($Al_2O_3$), titanium dioxide ($TiO_2$)), NSs made from semiconductor materials (e.g., silicon, germanium), polymeric NSs (e.g., nanospheres, nanocapsules), and lipid-based NSs (e.g., solid lipid nanoparticles (SLNs), NLCs) [72–75].

#### 4.3.1.2 Concentration of Starting Materials

The particle size of NSs is impacted by the concentration of starting material [75]. The nucleation rate increases with an increase in the concentration of the limiting reagent, and hence the reaction rate increases due to faster supersaturation [76].

#### 4.3.1.3 Type of Solvent

Solvents selection is an important step for NS synthesis methods as it impacts the reaction rate as well as the morphology of synthesized NSs. Even at uniform temperatures and time of reaction, the properties of NSs like growth pattern, shape, size, and size distribution can all be impacted by the solvents used during the synthesis [77].

### 4.3.2 Method of Synthesis

NSs can be synthesized by physical, chemical, biological, and hybrid techniques. Conventional physical and chemical synthesis results in higher product yield but they also lead to the production of toxic by-products. However, biological methods include biotechnological tools that utilize nontoxic precursors like algae, plants, fungi, yeast, bacteria, and isolated sugars. Also, biological synthesis is cost-effective as compared to physical and chemical methods [78, 79].

### 4.3.3 Reaction Parameters

#### 4.3.3.1 pH

The pH of the reaction media can affect the size, morphology, crystal structures, optical properties, and particle size distribution of the NSs being prepared [80, 81]. Studies have shown changes in the shape and size of silver and gold NPs at different

pH levels [74, 80]. Certain precursors, once broken down, may stimulate the creation of cations and anions, such as ammonium and hydroxide ions, which may alter the pH of a reaction medium [81].

#### 4.3.3.2 Temperature

Temperature is an important factor that affects all three methods of NSs synthesis. The required temperature for chemical synthesis should be less than 350°C while for physical synthesis it can be more than 350°C, while green technology needs temperatures less than 100°C [78]. Temperature variations can influence the nucleation, growth, and morphology of NSs [79]. The size of NSs can be increased by increasing the reaction temperature [72]. An increase in temperature leads to an increase in molecular energy that causes faster intermolecular collisions, hence enhancing the reaction rate [81]. However, elevated temperature may also lead to aggregation or undesired growth. Thus, regulating the temperature is critical for NSs synthesis [79].

#### 4.3.3.3 Pressure

Pressure is another reaction parameter that can affect the morphology of the NSs [81]. The rate of reduction of metal ions employing biological agents has been reported to be significantly quicker under ambient pressure conditions [78].

#### 4.3.3.4 Time

Reaction time determines the quality of synthesized NSs [78]. With an increase in reaction time, the aggregation of NSs increases, morphological changes occur, and structural size is greater [79, 81].

### 4.3.4 Environment Factors

The surrounding environment (presence of air, humidity levels, etc.) has a crucial influence on the physical structure and chemical character of the produced NSs. For example, the crystalline nature of the zinc sulfide NPs changes when the moisture was removed from the environment [78].

### 4.3.5 Post-Treatment

Post-treatment is done to remove impurities from the synthesized products. Calcination or annealing processes are done for post-treatment of NSs. The duration of post-treatment and temperature employed for it can impact the size, shape, morphology, crystallinity, and impurities in NSs [81].

## 4.4 CHARACTERIZATION TECHNIQUES FOR SYNTHESIZED NSs

Characterization techniques of synthesized NSs are important for the detailed study of the properties like their dimensions, morphology, composition, and surface characteristics. Though it is challenging to accurately characterize the NSs, but the selection of appropriate techniques can help [78]. The essential characterization techniques employed in the investigation of NSs are depicted in Figure 4.5.

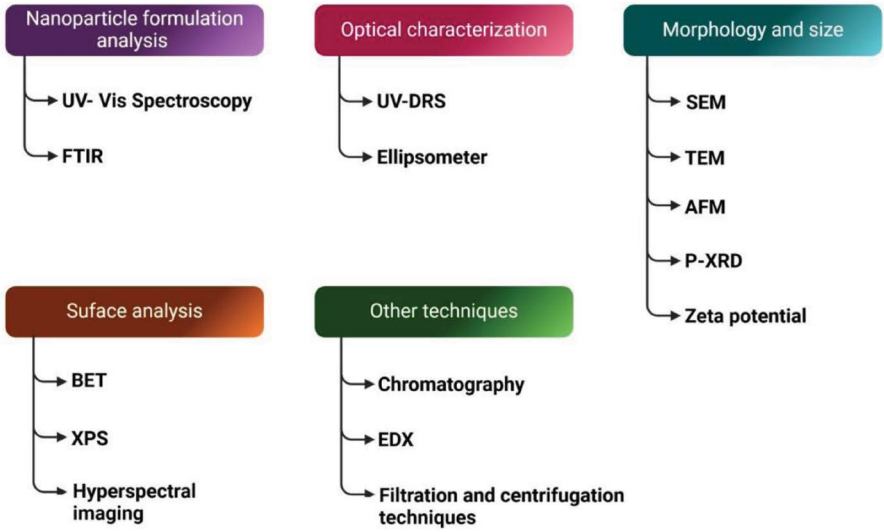


**FIGURE 4.5** Characterization techniques for NSs. The techniques are mostly employed to evaluate features such as size, morphology, crystallinity, surface area, and chemical composition of NSs.

### 4.4.1 Nanoparticle Formation Analysis

#### 4.4.1.1 Ultraviolet-Visible (UV-vis) Spectroscopy

UV-vis spectroscopy can confirm NSs synthesis by measuring plasmon resonance. Metal NPs exhibit distinct characteristic absorption bands (e.g., silver NPs show a peak between 400 and 450 nm, and gold NPs show a peak between 500 and 550 nm) [78]. The evidence for NSs stability can also be seen in UV-vis spectra (e.g., color change when silver NPs are synthesized due to the reduction of silver metal ions) [79].

#### 4.4.1.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy can determine the functional groups present on the surface of NSs by analyzing the molecular vibrations. Since each type of NP comprises a unique mix of atoms, we can identify functional groups contained inside the NPs and interactions between the NPs and surrounding molecules based on the FTIR spectra [78, 79].

### 4.4.2 Morphology and Size

Morphology and particle size distribution of NSs can be quantified using microscopic methods like transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM). Small particle size provides a higher interaction surface but may also cause aggregation of particles, so it is important to study particle size distribution [76, 78].

#### 4.4.2.1 Scanning Electron Microscopy (SEM)

SEM is used to evaluate the morphology of NSs by direct observation. It provides limited information about the size distribution, consumes more time, and is costly. The NSs solution is dried to produce a powder which is examined after coating with a conductive metal and the signals obtained, after exposure to the high-energy electron beam, reveal morphology, crystallinity, size of the NSs in the form of three types of images: external X-ray maps, backscattered electron images, and secondary electron images [78].

#### 4.4.2.2 Transmission Electron Microscopy (TEM)

TEM is also used to evaluate the morphology and size of NSs and has more resolution than SEM but the sample preparation for TEM is difficult and time-consuming as the sample should be ultrathin. Due to its higher-resolution property, it can be used for atomic-level study [79]. The NSs solution is dropped on the carbon-coated copper grid and dried under a mercury lamp, followed by exposure to a monochromatic beam of electrons that penetrates the sample and is projected onto a viewing screen to form an image. High-resolution TEM can determine the arrangement of the atoms and hence their crystal lattice, lattice vacancies and defects, screw axes, and surface atomic arrangement of crystalline NPs [78].

#### 4.4.2.3 Atomic Force Microscopy (AFM)

AFM is used to determine the dimensions and morphology of NSs. Unlike SEM and TEM, this technique has higher resolution as samples are scanned at submicron level using a probe tip [74, 78]. The NSs solution is dropped on a glass cover slip mounted on AFM stub and dried with nitrogen gas. AFM generates three-dimensional pictures; hence volume and height of NSs can also be analyzed [78].

#### 4.4.2.4 Dynamic Light Scattering (DLS)

DLS is one of the fastest ways to detect particle size distribution as it measures the Brownian diffusion coefficient by monitoring the motion-induced scattering of laser light to compute particle size [79]. As a monochromatic beam of light is focused onto a solution of spherical particles in Brownian motion, a Doppler shift occurs as the light strikes the moving particles, therefore altering the wavelength of the incoming beam of light by a value linked to particle size. However, the interpretation of DLS data becomes problematic when multiple scattering is involved, restricting its application in particular systems [79].

#### 4.4.2.5 Powder X-Ray Diffraction (P-XRD)

P-XRD identifies and measures distinct crystalline shapes or elemental compositions of NPs. It can detect distinct crystalline forms or the elemental makeup of NSs by measuring the angle of diffraction when X-ray beams are impinging on the sample. Particle size can also be calculated based on the breadth of the X-ray peaks using the Scherer formula [78]. P-XRD is performed effectively in both single and multiphase NSs identification. The appropriate comparison of the diffractograms of synthesized NSs with their physical mixes is necessary to acquire reliable information. Comparison of computer-calculated structural model of NSs with actual XRD spectra is the best technique to achieve excellent contrast [72, 79].

### 4.4.3 Surface Analysis

#### 4.4.3.1 Zeta Potential

Zeta potential can determine the stability and surface charge of the colloidal NPs, and type of components contained inside the NSs or coated on the external surface. The analysis is done using a Zetasizer nanomachine which analyzes the potential difference between the outer Helmholtz plane and the surface of shear. Either high positive or negative zeta potential values should be attained to maintain stability and minimize particle aggregation [78, 79].

#### 4.4.3.2 Brunauer Emmett Teller (BET) Analysis

BET is used to calculate the surface area of NSs and it is based on the adsorption and desorption principle and the Brunauer–Emmett–Teller (BET) theorem. Normally nitrogen gas is utilized as adsorbing material [72]. BET tests use a physisorption-based adsorption isotherm and the potential of a gas to be weakly bound to the external surface of NSs. Physisorption is a reversible process, and so gas easily adsorbs and desorbs to the surface of NSs. The amount of gas adsorbed at a series of different pressures, at a constant temperature, is calculated to give the adsorption isotherm [82].

#### 4.4.3.3 X-Ray Photoelectron Spectroscopy (XPS)

XPS is a surface-sensitive approach used to analyze the mechanism of reaction that happens on the surface of magnetic NPs, assess the bonding qualities of the different elements involved, and confirm the structure, elemental ratio, and exact bonding type of the elements contained in the magnetic NPs [72, 76].

#### 4.4.3.4 Hyper-Spectral Imaging

Hyper-spectral imaging is used to analyze the transformation of NSs in water samples and define the unique surface chemistry. Data defining sample information are supplied as geographic distributions and spectral characteristics specific to each NP type at the sensitivity of a single NP (size <10 nm) [78].

### 4.4.4 Optical Characterization of NSs

Optical characterization offers information regarding the absorbance, reflectance, luminescence, and phosphorescence of NSs [72].

#### 4.4.4.1 UV-vis Diffuse Reflectance Spectrometer (DRS)

DRS can be employed to measure optical absorption, transmittance as well as reflectance and is used to study band gaps in NSs. Band gap energy increases with a decrease in the size of NSs and draws conclusions about the photoactivity and conductance of the material [72]. The advantages of DRS are the vast availability, cost-effectiveness, faster analysis, and no requirement for specially trained operators and the disadvantage is shown by the varying band gap energy for the same sample in the literature [83].

#### 4.4.4.2 Ellipsometer

An ellipsometer is a non-destructive optical technique that determines the refractive index and extinction coefficient for NSs [72]. For ellipsometry thin films of NSs

are prepared and properties like layer thickness, grain size, crystallinity, and film surface morphology are determined. The crystallinity in ellipsometry is defined as the dielectric function between the single-crystalline and the amorphous form [84].

### 4.4.5 Other Techniques Used in Nanotechnology

#### 4.4.5.1 Chromatography

Chromatography is a non-destructive, sensitive, and fast technique to separate NSs present in solution based on their affinity to the mobile phase. Size exclusion chromatography is the best known technology for separating NSs based on size. This rather wide separation range allows NPs of various sizes to be detected in diverse environments and is particularly beneficial for monitoring the development of aggregates [78].

#### 4.4.5.2 Energy Dispersive X-Ray Spectra (EDX)

EDX is used to identify the elemental makeup of the NPs. X-rays are released from NPs after being attacked by an electron beam. An EDS detector that may be mounted to a scanning electron microscope can measure the number of X-rays that are released to balance the energy difference between two electrons. The energy of the produced X-ray is a distinguishing attribute of the element, and the element is consequently examined qualitatively and quantitatively [78].

#### 4.4.5.3 Filtration and Centrifugation Techniques

Centrifugation and filtering methods are two significant techniques applied for the preparative size fractionation of NPs. These approaches are cost-effective and very efficient. Preparative ultracentrifugation is utilized to pellet tiny particulate fractions for the separation and harvesting of aquatic colloids or NPs on TEM and AFM surfaces [78]. In the case of agglomeration and hydrophilicity, DLS can't give precise measurement, thus high-resolution technique of differential centrifugal sedimentation (DCS) can be employed [72].

## 4.5 CONCLUSION

NSs are playing significant importance in diverse areas such as medicine, cosmetics, environmental science, electronics, energy, and more. The size and shape of these NPs have a noteworthy impact on their physicochemical properties. Two techniques mainly bottom-up and top-down are utilized for the synthesis of different NSs. The selection of a suitable fabrication technique is required to achieve the NSs with desired physicochemical properties. Various factors showed a significant impact on NSs synthesis. Diverse factors influence the NSs synthesis including material nature, type of manufacturing method, and reaction condition. The characterization of NSs is of vital importance to ensure its formation, size, surface morphology and charge, stability, etc. Different techniques like Uv-vis spectroscopy, FTIR, TEM, SEM, AFM, DLS, P-XRD, etc. found to be promising in the characterization of NSs. Although there has been a lot of research on NSs, further advancements in NSs fabrication and characterization techniques are essential to fully unlock their potential and enhance their applications.

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# 5 PEGylation and Other Stealthing Techniques

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## 5.1 INTRODUCTION

PEGylation is a process that involves attaching polyethylene glycol (PEG) chains covalently to molecules, such as proteins, drugs or bioactive substances, to improve their circulation properties [1, 2]. It is a well-established strategy for improving the target specificity, circulation time, and stability of liposomes, thereby improving their stealth properties. Protein and peptide drugs hold great promise as therapeutic agents. However, many are degraded by proteolytic enzymes, can be rapidly cleared by the kidneys, generate neutralizing antibodies and have a short circulating half-life. It is the process by which PEG chains are attached to protein and peptide drugs, can overcome these and other shortcomings. By increasing the molecular mass of proteins and peptides and shielding them from proteolytic enzymes, it improves pharmacokinetics. Drugs can overcome these and other shortcomings by increasing the molecular mass of protein and peptides and shielding them from proteolytic enzymes, it improves pharmacokinetics [3].

PEG (Figure 5.1) is authorized by the FDA to be used as a vehicle or base in injectable, topical, rectal, and nasal formulations of medicines, foods, and cosmetics [4]. It is not immunogenic and has very low toxicity. Abuchowski and colleagues initially reported this method in 1970 using the PEGylation of albumin and catalase [5]. To increase the duration of their blood circulation, liposomes undergo PEGylation. PEGylated liposomes have a longer half-life, reduced plasma clearance, and a distribution shift toward sick tissues compared to their classical counterparts. It forms a hydrated shell integrated into the liposome's lipid bilayer, shielding it from protein degradation. Additionally, these are less prone to leak medication while in circulation and are not as widely absorbed by the reticuloendothelial system (RES) [6, 7] (Figure 5.2).

In contrast to other technologies, the API in PEGylated products undergoes a long-lasting chemical modification, releasing the medication as a permanent action

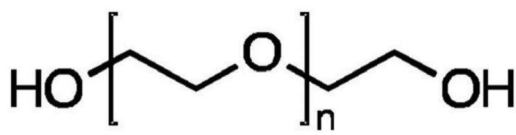


**FIGURE 5.1** Structure of a single PEG chain.

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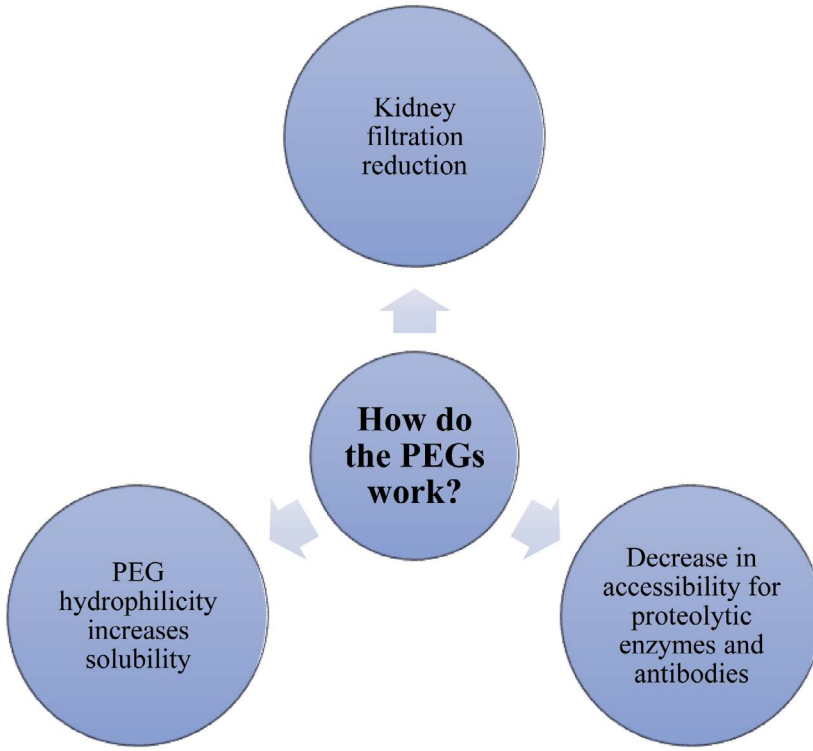



**FIGURE 5.2** How do the PEGs work?

rather than a formulation. As a result, the product is classified as a new API. The formulation process is reversible for other prepared products, like tablets and liquid capsules; the medicine becomes active after being released from the formulation, while the API stays the same [8–10]. Various types of PEG derivatives used for PEGylation in Table 5.1.

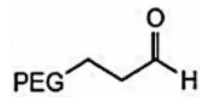
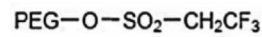
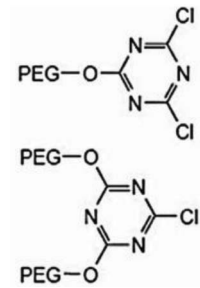
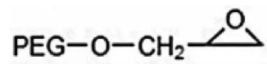
Reducing kidney filtration: The conjugated drug compound's apparent size is greatly increased by PEGylation. The renal glomerular capillary typically measures between 6 and 12 nm in diameter. However, the PEG-protein conjugate's apparent hydrodynamic radius, which limits the formulation's outward reach, is about 20 nm [11].

This concise overview sheds light on the makeup of PEGylated liposomes and the properties—such as surface density, molecular weight (MW), the presence of linkers and acyl groups, etc.—that determine their functioning. The clinical implications of the physicochemical methods used to test the stability and characterize the PEG liposomes are also covered. This article gives readers a comprehensive overview of the different types of PEGylated lipids, how to determine how stable they are in liposomal formulations, and the latest advancements in PEGylated liposomal formulation development [19].

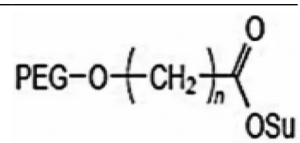
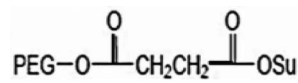
When PEG is attached to nanolipid drug carriers, the immune system—particularly monocytes and macrophages—is unable to identify and remove the medication,

## TABLE 5.1
## Derivatives of PEG

**PEG Derivatives That Maintain the Charge of the Native Protein in the Final Conjugate**

| Sl. No. | Alkylating PEGs | Properties | Structure |
|---|---|---|---|
| 1 | PEG—aldehyde (also in the form of more stable acetate) [12] | A two-step reaction; the first product (a Shiff base) is reduced by $NaCNBH_3$ when the coupling reaction is carried out at low pH = 4.5–5, it labels only the α-amino group. |  |
| 2 | PEG-tresyl or tosyl [13] | Not much used because the chemistry leads to a mixture of products. |  |
| 3 | PEG—dichlorotriazine or chlorotriazine [14] | Now they are abandoned for therapeutic application because of their toxicity. |  |
| 4 | PEG—epoxide [15] | Slowly reactive, rarely used. |  |

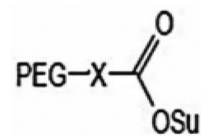
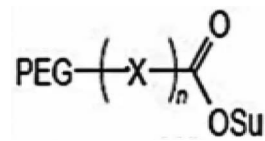
**PEG Derivatives That, After Amino Coupling, Lead to a Loss of Positive Charge in the Final Conjugate with Respect to Starting Protein**

| | | | |
|---|---|---|---|
| 5 | PEG-carboxylates [16] | The carboxylic group is activated as N-hydroxy succinimidyl ester, imidazole, or benzotriazole. The kinetic rate of conjugation depends on the numbers and eventual ramification of $CH_2$ groups linked to the carboxyl group. |  |
| 6 | PEG-succinimidyl succinate [17] | The ester bond between succinic acid and PEG is easily hydrolyzed |  |

*(Continued)*

**TABLE 5.1** *(Continued)*
**Derivatives of PEG**

**PEG PEG Derivatives That, After Amino Coupling, Lead to a Loss of Positive Charge in the Final Conjugate with Respect to Starting Protein**

| Sl. No. | Alkylating PEGs | Properties | Structure |
|---|---|---|---|
| 7 | PEG-amino acid-succinimidyl ester [17] | Nle or ßAla as amino acid moiety allows an easy quantification of the number of linked PEG chains by amino acid analysis. |   |
| 8 | PEG-peptide-succinimidyl ester [18] | This allows the removal of PEG by CNBr treatment for an easy localization of PEGylation site. Lysosomal cleavable sequences, as H-Gly-Phe-Lue-Giy-OH, allow the release of the bound drug inside the cell. |   |

prolonging its time in the bloodstream. By protecting the carriers from opsonins—proteins that mark foreign particles for phagocytic cell clearance—the PEG coating enables the medicine to circulate for noticeably longer periods of time than uncoated carriers [20, 21]. Pharmaceutical businesses use PEG with a MW greater than 20 kDa to provide a "stealth" effect that reduces immune system detection and extends the circulation of drugs or nanoparticles (NPs) [8, 22]. The surface modification strategies for liposomes are provided in Figure 5.3.

## 5.2 TECHNIQUES FOR PEGylation

Pre-insertion and post-insertion are the two main methods depending on the manufacturing process that can be used to PEGylate NPs. Every one of these approaches has unique traits, benefits, and things to keep in mind.

**Pre-Insertion Technique**: PEGylation takes place during the NPs' production in the pre-insertion process. PEG chains can be directly integrated into the structure of the NPs because they are a part of the formulation or reaction mixture. Prior to the formation of the NPs, PEG is added to the reaction mixture. Through chemical processes, it can form a covalent bond with the NPs during their creation. It offers homogeneity, which makes all NPs more

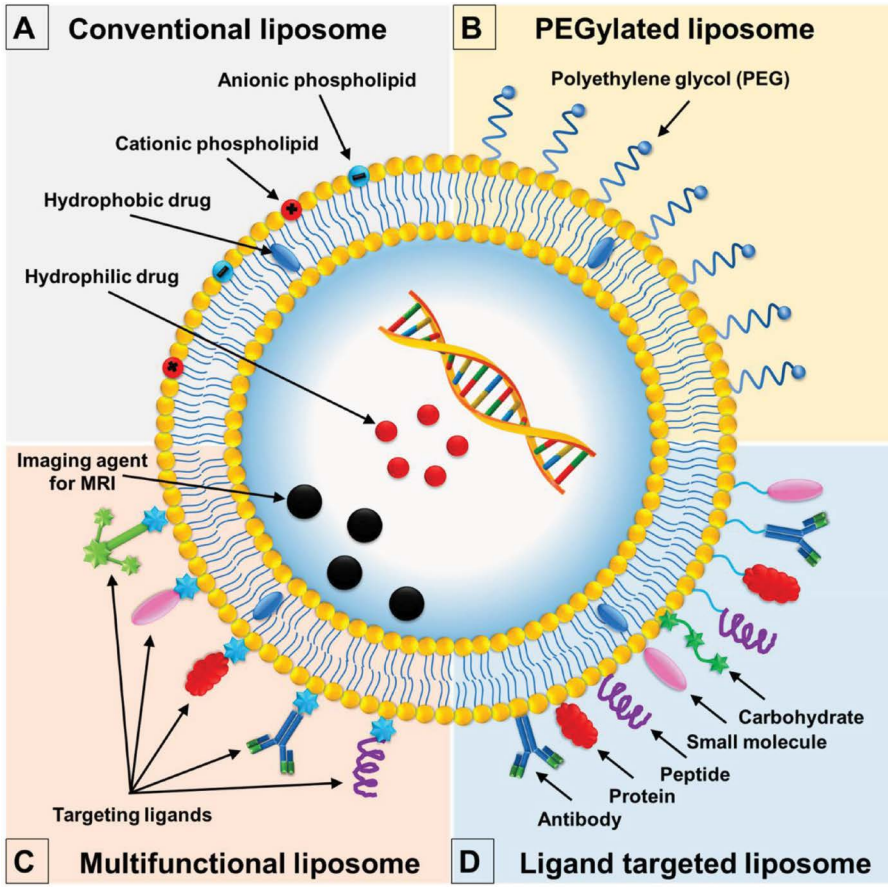



**FIGURE 5.3** Surface modification strategies for liposomes. (Reproduced with the permission from Ref. [23].)

uniformly PEGylated and produces consistent qualities. Additionally, it creates more stable NPs with incorporated PEG chains. Because there is no need for an additional post-insertion phase, the procedure can be made simpler. Similar to how adding PEG during synthesis might make optimization more difficult since it may alter particle formation and characteristics, there are steps that can be taken to make optimization less hard. If PEG alters the release profile of the NP, there may be a chance for a less regulated release of the medication [24].

**Post-Insertion Technique:** In this case, PEGylation takes place subsequent to the synthesis of the NPs. The pre-formed NPs are supplemented with PEG chains. PEG molecules, frequently in the form of reactive PEG derivatives, are added to the NPs after initial synthesis in order to alter their surface. It provides versatility that enables surface qualities to be tailored

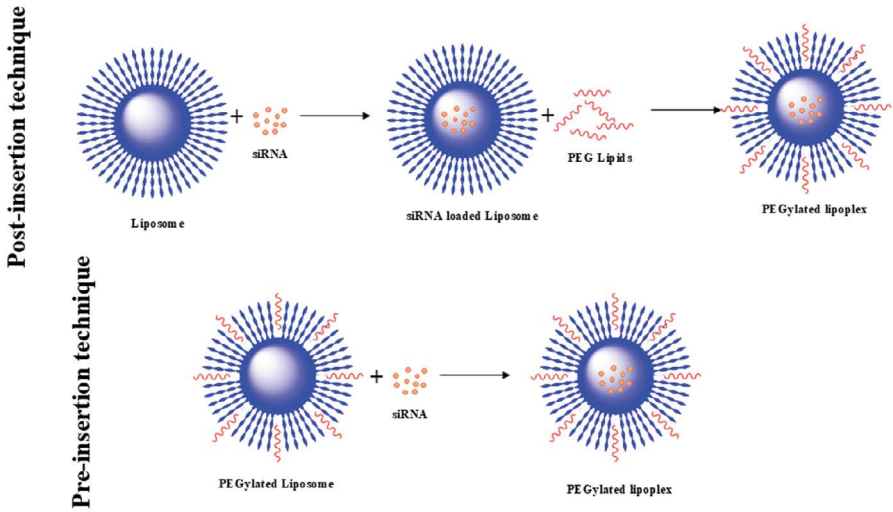



**FIGURE 5.4** Techniques of PEGylation. (Reproduced with the permission from Ref. [26].)

after NPs creation, allowing customization according to particular applications or desired features. Controlling the quantity of PEG added in the post-insertion process makes adjusting the PEG density simpler. Since the change takes place after production, the encapsulated medication can frequently be kept better. Inconsistencies could result from some variation in the PEGylation levels of the different NPs. To guarantee effective PEG attachment while preventing NP destruction, reaction conditions must be carefully optimized [25].

All things considered, pre-insertion entails incorporating PEG into the NP matrix during the synthesis process, which promotes homogeneity but could make synthesis optimization more difficult. Although it is unlikely, post-insertion entails altering the surface of the NPs with PEG after synthesis. This permits customization and control over surface characteristics, but it may lead to variation among the NPs. Both methods are useful, and which one is used will rely on the particular objectives of the formation of the NPs and the planned use.

The techniques of PEGylation are summarized in Figure 5.4. PEGylation can be divided based on site of PEGylation as follows:

### 5.2.1 Covalent PEGylation

- Amine-Reacting PEGs: Reagents such as NHS-PEG or maleimide-PEG can be used to link PEG to amine groups on proteins or peptides. This is among the most widely used PEGylation techniques [27, 28].
- Carboxylic Acid-Reacting PEGs: By employing carbodiimide chemistry, PEG can be coupled to carboxylic acid groups, improving its solubility and stability [29].

### 5.2.2 Site-Specific PEGylation

- Enzyme-Mediated PEGylation: Sortase and transglutaminase are two examples of enzymes that can help PEG bind to particular protein locations, resulting in more regulated and predictable processes [29].
- Genetic engineering: By adding particular amino acids to a protein's sequence, distinct reactive sites for PEG attachment can be produced [29].

### 5.2.3 Non-Covalent PEGylation

- Physical Encapsulation: PEG provides an alternative method of enhancing drug delivery and stability by enabling the creation of liposomes or NPs that encapsulate medications without the need for direct covalent bonding [30].

### 5.2.4 Dual-PEGylation

- Multiple PEG chains can be added to a single molecule to increase solubility and decrease immunogenicity, but maintaining the molecule's biological activity requires careful attention [31].

## 5.3 REVIEW OF PEGylated LIPIDS

The lipid molecules self-assemble to form liposomes when an aqueous medium is present. They are composed of a hydrophilic head group and a hydrophobic tail area [32]. In contrast to metal, polymer, and dendrimer-based carriers, they are perfect candidates for vaccines and drug carriers because of their hydrophilic aqueous core and hydrophobic lipid bilayer area, which allow them to encapsulate both hydrophobic and hydrophilic molecules. In order to enhance the biodegradability, biocompatibility, and versatility of liposomes for possible uses in the encapsulation of medications used in cancer therapy, their properties can be adjusted by adding polymers, cholesterol, or membrane proteins, as well as by selecting different lipids and size techniques [33]. In addition, the size, charge, and number of layers of liposomes can be altered based on the intended use. Conventional and long circulation liposomes are the two main categories into which liposomal drug delivery falls. In the early 1980s, conventional or first-generation liposomes were created for commercial use in the administration of hydrophobic doxorubicin and amphotericin. Together with cholesterol, these liposomes contained a mix of cationic, anionic, and zwitterionic phospholipids [34].

However, it was discovered that plasma proteins—also known as opsonin proteins—were drawn to traditional liposomes from the bloodstream. For macrophages, the opsonins act as identifying markers. Opsonins cling to the lipid surface via electrostatic forces. Because opsonins are present on the liposomal surface, they are vulnerable to macrophage invasion and, ultimately, RES clearance [35, 36]. Another name for this phenomenon is liposomal opsonization. Several techniques have been used to make liposomes stealthy in order to stop them from opsonizing and to prolong their circulatory circulation. One popular method is to introduce steric stabilization by adding hydrophilic polymers to the liposomal surface. This

illustrates how steric stabilization affects the clearance of RES; opsonins only stick to regular liposomes and not to the sterically stabilized liposomes. This causes the macrophage cells to arrest the conventional liposomes, which are then cleared by the RES. Stealth liposomes, on the other hand, are able to reach the tumor locations and have longer circulation durations. Longer circulation times are caused by the liposomes' stealthy nature. The polymer's density, molecular weight, spatial conformation, and hydrophilicity all influence its stealthiness [24].

Polysaccharides [37], polyvinyl alcohol (PVA) [38], polystyrene (PST) [39], polylactic acid (PLA) [40], polyacrylamide [41], and PEG [42, 43] are some of the commonly reported polymers; however, because of their carboxy groups, PLA and PST have zeta potentials ranging from –20 to –76 mV, making them highly anionic, while polysaccharide coatings, on the other hand, activate the immune system due to the presence of hydroxyl groups, leading to elimination [44]. Liposomes coated with PVA and polyacrylamide copolymers have been observed to experience mechanical degradation and subsequent content leakage. Among the different polymers analyzed, PEG molecules have shown potential in steric stabilization because they are hydrophilic, biocompatible, nonimmunogenic, and uncharged.

The benchmark formulation that exemplifies PEG's stealth is Doxil™. Because of its hydrophilicity and uncharged surfaces, PEG is considered stealthy. PEG prevents opsonization by increasing the hydrophilicity of the liposomes and decreasing the zeta potential or total charge on their surface. This feature has been shown in earlier research where PEG coating on anionic liposomes increased circulation by lowering the zeta potential to a value close to neutral [45].

PEG chains, a linker, and a hydrophobic anchor make up PEGylated lipids [46]. In PEGylated lipids, the linker connects one end of the PEG chain to the hydrophobic anchor. PEG chains can be functionalized by covalent conjugation with folate, biotin, amine, azide, and carboxylic acid [47]. Their MW typically ranges from 50 kDa to 400 kDa. PEG lipid functionalization, which is essential for improving target specificity, has been widely documented and is outside the purview of this review. The conformation of PEG chains on the surface of the lipid bilayer is influenced by the MW and grafting density. The PEG chains expand and branch out to engage with nearby molecules as the grafting density rises, whereas the PEG molecules assume a less interactive mushroom regime with conforming chains at lower grafting densities. In order to enhance a liposome's stealth characteristics, the brush regime is recommended because it involves less particle contact and diffuses through tissues more quickly than the mushroom regime. Furthermore, hydrophobic and electrostatic interactions are the main ways that opsonins bind. Nonionic PEG chains in brush form make the liposome's surface hydrophilic, which lessens interactions [48, 49]. Additionally, some opsonins that have been adsorbed have a tendency to compress the brush-like chains into a mushroom-like shape. The attracting properties of opsonins are prevented from reaching the liposome surface by this energy shift.

Alexander-deGennes' theoretical model of polymer regimes states that the length of the random PEG coils (Rf) and the distance (D) between the grafting sites determine when the polymer regime changes from mushroom to brush. The mathematical representation of the Rf or Flory dimension is $aN^{3/5}$, where a is the size of the monomer and N is the degree of polymerization [50].

PEG chains envelop the liposomes in a fixed aqueous layer thickness (FALT) in all conformations. Liposome circulation time and regime type are regulated by this FALT value [51]. According to reports, extending the circulation period can be achieved by combining PEG chains of varying lengths. Through linker moieties, the PEG chains are further joined to the hydrophobic acyl anchor. Phosphate ester, ether, disulfide, carboxyl ester, amide, and peptide connections are examples of common linker moieties [52]. The liposomes' surface charge and binding characteristics are influenced by the linker moieties. PEGylated lipids are classified as PEG-phospholipids or PEG-non-phospholipids according to whether the traditional linker, phosphate linker, is present or absent. PEG chains branch into the aqueous zone, and the hydrophobic anchor, which is the third component of PEG lipids, is made up of acyl groups that attach to the lipid bilayer. The degree of PEGylated lipid inclusion in the liposome and the lamellar or micellar shape of the lipid assemblies are determined by the acyl groups [53].

A fatty acid chain, such as distearoyl, dipalmitoyl, or dimyristoyl, is often the acyl group in PEG-phospholipids. It is covalently attached to the polar phospholipid head group. Following that, these head groups are joined to a linear chain of methoxy-PEG (mPEG) [54]. It is discovered that the FALT values, which in turn determine the circulation duration, are influenced by the length of the mPEG chains, which is directly connected to the WR. Because of their natural mushroom shape, shorter m-PEG chains (350–750 Da) have demonstrated very few effects in preventing macrophage clearance. It has been demonstrated that lengthening the mPEG chain length lengthens the circulation time. Furthermore, owing of their varying hydrophilic and hydrophobic characteristics, mPEG phospholipids—that is, 1,2-distearoyl-snglycero-3-phosphoethanolamine-PEG—that contain a combination of PEG chain lengths in the same molecule lengthen circulation duration and have longer tumor contact times. It has also been discovered that the amount of mPEG-phospholipids in a liposome affects how quickly medicines are released. Diffusion-controlled drug release is changed to interfacial-controlled drug release by the higher MW mPEG-phospholipids [55].

The complement system, which controls innate immunity, can be inhibited and hypersensitivity reactions can result from neutralizing these anionic charges with cationic groups or methylation. Typical phosphate linkers have an anionic character. The costs associated with the production of phospholipids are not justified because of their vulnerability to enzymatic degradation by lipases and phospholipases, which results in their rapid clearance [56].

Longer acyl chained ceramides (C24) had stronger anchoring properties than shorter chain ceramides (C8), which led to longer circulation times and higher drug release rates, according to a study examining the effects of different PEGylated ceramide lengths on liposome circulation times [57].

Cholesterol serves as the hydrophobic anchor in cholesterol-linked PEG, also known as cholesterol-PEG (Chol-PEG). Because of its lipophilicity, compatibility with other lipids, and stabilizing qualities, cholesterol is a good option for using ester bonds to anchor PEG chains. Chol-PEG has been shown to control membrane fluidity, which strengthens the bilayer and stops medication leakage. Furthermore, it is discovered that the percentage of Chol-PEG affects how well the medication loads. More

medications could be encapsulated by the formulation with lower Chol-PEG ratios [58]. According to studies, cholesterol can be linked with succinate and carbamate linkers to create pH-cleavable PEG chains. Chol-PEG showed a shorter circulation duration than PEG phospholipids, despite its benefits. This is because the benefits provided by PEG chains are negated by cholesterol, which binds deeply into the hydrophobic zone of the lipids. It is suggested that additional linkers be added to get around this restriction, however doing so makes the synthesis process more difficult [59].

Additionally, to improve drug absorption, the PEG chains must be broken when the circulation time is reached. Attaching linker moieties that either cleave the PEG chains when they reach the target spot or when they are exposed to an appropriate stimulus—such as a change in pH, temperature, or the presence of an enzyme in the cellular microenvironment—is how this is done. Vinyl ether, hydrazine, disulfide, peptide, and ester bonds are a few common moieties that are employed as cleavers. Vinyl ether bonds are labile in acidic environments, particularly when the pH is less than 5, because they are non-reactive in near neutral and basic conditions [60]. Under different pH levels, vinyl ether-linked PEG to dioleoyl phosphatidyl ethanolamine (DOPE) liposomes have shown effective release of medicinal contents following PEG moiety cleavage. Additionally, it has been noted that reactive singlet oxygen produced by photosensitizers can cleave these PEG conjugated vinyl ether bonds.

Peptide bonds can only be cleaved in the presence of tumor-specific enzymes, such as matrix metalloproteinases (MMPs). According to *in vitro* research, peptide-linked PEG lipids prefer to collect on tumor sites and have prolonged circulation durations because of the enhanced permeability and retention effect (EPR), which results from the tumor tissue's inability to drain [61]. PEGylated liposomes conjugated with disulfide bonds are preferred for intracellular delivery because glutathione, an antioxidant that is overexpressed in the cytoplasm of malignant cells, cleaves the disulfide linkage in nanocarriers. Additionally, a number of studies suggest that the delivery of antibodies via cleavable PEG connected by a disulfide connection may be utilized in the diagnosis and therapy of cancer [62].

Based on the available literature, the Table 5.2 summarizes FDA-approved formulations involving PEGylated liposomes.

### 5.3.1 Experimental Methods for Evaluating PEGylated Liposome Stability

When designing vaccine and drug carrier systems based on liposomes, thermal and physical stability are crucial considerations. PEGylated liposomes' physicochemical characteristics and stability are being evaluated using a range of analytical and optical techniques in preparation for *in vivo* research and clinical trials. To clarify the structure and characteristics of the liposomes, some investigations have combined a number of methods, including nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), differential scanning calorimetry (DSC), and cryogenic electron microscopy (EM). These investigations shed light on the liposomes' steric and thermal stability. However, methods like FTIR, EM, and NMR are highly sample-specific and necessitate a complex and specialized setup. Conversely, DSC and DLS are rather straightforward methods that still yield the required data regarding sample stability [81].

**TABLE 5.2**
**FDA-Approved PEGylated Liposomal Formulations**

| Sl No. | Formulation Brand Name | Encapsulated Drug | Lipids/Excipients | Therapeutic Indication | Clinical Stage | Company |
|---|---|---|---|---|---|---|
| 1 | Marqibo [63] | Vincristine | Sphingomyelin+cholesterol | Acute lymphoblastic leukemia | Approved | Talon Therapeutics, Inc. |
| 2 | Doxil [64] | Doxorubicin hydrochloride | HSPC:cholesterol:DSPE-PEG | AIDS-related Kaposi's sarcoma, various cancers, such as ovarian/breast cancer | Approved | Sequus Pharmaceuticals (1995) |
| 3 | Onivyde [65] | Irinotecan hydrochloride trihydrate | DSPC: Cholesterol:DSPE-PEG | Pancreatic adenocarcinoma | Approved | Merrimack Pharmaceuticals Inc. (2015) |
| 5 | ThermoDox [66] | Doxorubicin | DPPC, myristoyl stearyl phosphatidylcholine and DSPE-N-[amino(polyethylene glycol)-2000] | Hepatocellular carcinoma and also recurring chest wall breast cancer, prostate cancer | Phase III | Celsion Corporation |
| 6 | 2B3-101 (2X-111) [67] | Doxorubicin | Glutathione PEGylated liposomes | Brain metastases and recurrent malignant glioma | Phase I/IIa | 2-BBB Therapeutic |
| 7 | 2B3-102 (ENX-201) [68] | Methylprednisolone | Glutathione PEGylated liposomes | Acute relapses of multiple sclerosis | Phase I | 2-BBB Therapeutic |
| 8 | S-CKD602 [69] | Potent topoisomerase I inhibitor (semi-synthetic analog of camptothecin) | MPEG-linked phospholipids | Various cancers like lung cancer, breast cancer, brain cancer, colorectal cancer, ovarian cancer, small cell lung cancer, and leukemia | Phase I | Alza Corporation |
| 9 | Lipoplatin [70] | Cisplatin | DPPG, soy phosphatidyl choline, mPEG-distearoyl phosphatidyl-ethanolamine lipid conjugate and cholesterol | Non-small cell lung cancer | Phase III | Regulon Inc. |

*(Continued)*

**TABLE 5.2** ***(Continued)***
**FDA-Approved PEGylated Liposomal Formulations**

| Sl No. | Formulation Brand Name | Encapsulated Drug | Lipids/Excipients | Therapeutic Indication | Clinical Stage | Company |
|---|---|---|---|---|---|---|
| 10 | MCC-465 [71] | Doxorubicin | DPPC, cholesterol and maleimidated palmitoyl phosphatidyl ethanolamine; immunoliposomes tagged with PEG and the $F(ab')_2$ fragment of human monoclonal antibody GAH | Metastatic stomach cancer | Phase I | Mitsubishi Tanabe Pharma Corporation |
| 11 | SPI-077 [72] | Cisplatin | Soybean phosphatidylcholine, cholesterol | Lung, head and neck cancer | Phase II | Alza Corporation |
| 12 | MM-302 [73] | Doxorubicin+ Trastuzumab | DSPC, Cholesterol, DSPE-PEG-2000 and DSPE-PEG-Maleimide or DSPE-PEG-NHS | Breast cancer | Phase I/II (Terminated) e 1/2 | Merrimack |
| 13 | INX-0125 [74] | Vinorelbine tartrate | Cholesterol and sphingomyelin | Advanced solid tumors | Phase I | Inex Pharmaceuticals |
| 14 | INX-0076 [75] | Topotecan | Cholesterol and sphingomyelin | Advanced solid tumors | Phase I | Inex Pharmaceuticals |
| 15 | Alocrest [76] | Vinorelbine | Sphingomyelin/cholesterol (OPTISOME™) | Breast and lung cancers | Phase I | Spectrum Pharmaceuticals |
| 16 | Camptosar [77, 78] (LE-SN38, LEM, LE-AON, LEP) | 7-ethyl-10-hydroxycamptothecin (active metabolite of irinotecan) | PC, cholesterol, and cardiolipin | Various solid tumors, prostate cancer, various solid tumors, various solid tumors | Phase I/II | NeoPharm Inc. |
| 17 | ATRA-IV [79] | Aroplatin | Trans-retinoic acid | Colorectal cancer, acute promyelocytic leukemia | Phase II | Antigenics Inc. |
| 18 | AmBisome [80] | Amphotericin B | Alpha tocopherol, cholesterol, DSPG-Na, HSPC | Fungal infection | Approved | Gilead Sciences |

In DLS, the Brownian motion of the liposomes in the solution causes a monochromatic light that is reflected in the liposomal solution to be scattered. The liposomes' diffusion coefficient is correlated to size. The measurement of size is also affected by the liposomal solution's temperature and viscosity [82]. The diffusion coefficient ($D_c$) is related to the hydrodynamic radius ($R_H$) is given by the stokes Einstein equation:

$$\begin{aligned} &Diffusion\ coefficient\ \left(D_c\right) \\ &= \frac{Boltzmann\,coefficient\ \left(kB\right)* Absolute\,Temperature\ \left(T\right)}{6\pi * Viscosity\,of\ aqueous\,medium\ \left(\eta\right)* Hydrodynamic\,radius\ \left(R_H\right)} \end{aligned}$$

where kB is Boltzmann coefficient ($1.380\times10^{-23}$ kg $m^2$ $s^{-2}$ $K^{-1}$), T is an absolute temperature, and η is the viscosity of the aqueous medium.

This method produces the liposomes' polydispersity index (PDI), which establishes the liposomes' size uniformity, in addition to the hydrodynamic radius measurements. The FALT, which is obtained from the zeta potential or surface charge of the liposomes, is another crucial factor that affects the PEG liposomes' capacity to circulate. The Laser Doppler electrophoresis method is used to measure the zeta potential. A coating of ions from the solution firmly adheres to the liposome surface to create a stern layer when PEGylated liposomes are dissolved in water and voltage is applied. Ions with opposing charges adhere loosely to this charged layer, which is known as the diffuse layer. The electrical double layer is the name given to the two layers taken together. The zeta potential is the electrical potential that is measured at the electrical double layer's surface [83].

When a liposomal suspension is exposed to an electric field, the liposomes migrate in the direction of the zeta potential measurement cell's oppositely charged electrodes. The Henry equation links the electrophoretic mobility ($U_e$), which is the ratio of the particles' velocity of motion to the electric field, to the zeta potential ($z$) which is shown below.

$$U_e = 2ezf(ka)^3 h$$

$U_e$ is the electrophoretic mobility
$\varepsilon$ is the dielectric constant
$z$ is the zeta potential
$f(\kappa a)$ is the Henry function
$\eta$ is the absolute zero-shear viscosity of the medium

Atomic force microscopy (AFM), fluorescence microscopy (FM), phase contrast microscopy (PCM), and cryo-transmission electron microscopy (cryo-TEM) can all be used to determine how PEG chains affect the orientation of the lipids in the liposome and other morphological features. Artifacts related to spatial and resolution can be produced by sample preparation in the cases of EM and AFM and the presence of additional dyes in FM. This lack of long-range order is caused by the difficulties in crystallizing the samples, which limit the use of X-rays to acquire information on the structural features [84].

When PEGylated lipid molecules undergo an endothermic thermal transition from gel to fluid crystalline, they exhibit distinctive pretransition and melting temperatures. The presence of molecules in the lipid bilayer alters the nature of the thermal transition. The area under the melting transition is used to calculate the change in the enthalpy of transition. The presence of surface-bound or encapsulated drugs is implied by the shift in the lipids' melting peak's form. By calculating the thermodynamic characteristics of the lipid-molecule system, more details on these molecules' cooperativity can be found [85]. It is evident from earlier discussions of the makeup, characteristics, and benefits of PEGylated lipid molecules that PEGylated formulations offer longer circulation times, preferential accumulation to sites of interest (like tumors), and shielding effects against macrophages. Numerous PEGylated liposomes have been evaluated for clinical trials and authorized by the FDA for patient treatment as a result of extensive studies in stealth technology [86].

In many formulations, PEGylated lipids are usually combined with cholesterol and ordinary phospholipids. Doxil® was the first PEGylated liposomal product to be approved. In 1995, Sequus Pharmaceuticals in the United States created the intravenous formulation Doxil® to administer DOX (doxorubicin) for the treatment of breast and ovarian cancer. This formulation contains hydrogenated soy phosphatidylcholine (HSPC, 59 mol%), cholesterol (39 mol%), and the phospholipid DSPE-PEG 2000 (5 mol%) [64, 87]. Subsequently, later, the FDA-approved Onivyde™, which contains irinotecan, another anticancer drug, to treat metastatic adenocarcinoma. In the ratio 3:2:0.015, Onivyde™ comprises cholesterol, distearoyl-sn-glycero-phosphoethanolamine (DSPC), and distearoylphosphatidyl ethanolamine (DSPR-PEG2000) [65]. Thus, the stealth nature of PEGylated liposomes makes them suitable for encapsulation of variety of PEGylated liposome therapeutics.

Several other formulations which contain PEGlyated phospholipids and Chol-PEG are currently in different phases of clinical trials as discussed in Table 5.2.

Combining PEGylated lipids with photopolymerizable lipids (e.g., $DC_{8,9}PC$ (1,2 bis (tricosa-10,12-diynoyl)-sn-glycero-3-phosphocholine)) is an intriguing new usage for PEGylated lipids. A polymeric lipid with a tubular shape is $DC_{8,9}PC$. In the past, $DC_{8,9}PC$ and DPPC have been employed in succession to release a photosensitizer (HPPH), which is presently undergoing clinical studies, when exposed to light. NanoRed™ is developing this unique technology for a range of therapeutic approaches [88].

According to a recent study, tubule-forming photopolymerizable lipid $DC_{8,9}PC$ can be given a stable vesicular morphology by using the PEGylated phospholipid DSPE-PEG2000. The creation of a lamellar structure can therefore be induced by the presence of hydrophilic PEG chains, which eliminates the need for DPPC and produces a binary lipid stealth nanosystem. At very high efficiency, the $DC_{8,9}PC$ formulations were able to include up to 20 mol% of DSPE-PEG-2000 and encapsulated HPPH. As far as we are aware, there has never been a lipid formulation that included such a high proportion of PEG lipids. The encapsulation of dexamethasone, a powerful medication for rheumatoid arthritis, in this formulation was also studied by another group. The use of $DC_{8,9}PC$ has been shown in both of these experiments to produce prolonged circulation, stealth, and preferential accumulation of these formulations in the animal locations of interest [89].

In conclusion, PEGylated liposomes are a desirable option for drug delivery applications due to their long circulation and stealth characteristics. Nevertheless, some

research has shown that PEGylated liposomes can cause an unanticipated immunological reaction. Accelerated blood clearance (ABC) is one such reaction; when PEGylated liposomes are administered on a regular basis, antibodies are produced against the carrier [90]. This has resulted in hypersensitive immunological responses and decreased therapy efficacy. Therefore, additional study is needed to comprehend the underlying mechanisms causing these reactions. Designing PEGylated liposomes that can be utilized for the duration of the treatment would be aided by this.

### 5.3.2 Other Stealthing Technologies

Other than PEG coating, there are some other reported biocompatible synthetic polymers used in stealth liposomes as mentioned in Table 5.3. HPMA polymer as stealth polymer can be described in Figure 5.5.

With the help of a water-soluble polymer called polyvinylpyrrolidone (PVP), polyaniline (PANI) with excellent processibility and stability was made in an acidic aqueous dispersion or solution. The stealth liposomes' high degree of dispersion, near solubility, and storage stability. Although un-polymerized acrylamide may exist in the polymerized acrylamide, polyacrylamide (PAM) or PAA is an easily cross-linked acrylate polymer made from acrylamide subunits. As a result, it is advised to handle it carefully. Because of its high water absorption capacity, it forms a soft gel that can be employed in processes like the production of stealth liposomes. It also serves as a suspending agent and thickener. An important class of polymers, poly (2-etyl-2-oxazoline) and poly(2-alkyl-2-oxazoline) have a wide range of potential uses, including as sensors, active agent carriers in drug administration, and thermosensitive materials. Additionally, they all show longer blood circulation times and less splenic and hepatic absorption. The stealthing qualities of TPGS (Figure 5.6) or d-α-tocopheryl PEG 1000 succinate, a vitamin E derivative that also functions as a solubilizer, stabilizer, and antioxidant, are demonstrated in some published papers [92].

## 5.4 BENEFITS OF PEGylation

- Extended Circulation Period: PEG chains reduce MPS absorption and considerably lengthen the liposomes' circulatory circulation period by forming a hydrophilic "shield" around them. More medication accumulation at the target site is made possible by this extended circulation. It prolongs the period that drugs are in the bloodstream by decreasing liver absorption and renal clearance, which can be termed as extended half-life [104].
- Reduced Immunogenicity: PEGylation reduces the liposomes' immunogenicity, reducing unfavorable immunological reactions. This is especially crucial when administering medication repeatedly [105].
- The EPR effect, which takes advantage of the tumors' poor lymphatic drainage and leaky vasculature, allows PEGylated liposomes to passively aggregate in tumor tissues. The effectiveness of treatment is increased by this passive targeting, which enhances drug delivery to the tumor site [106].
- Increased Solubility: Enhances the solubility of poorly water-soluble drugs [107].
- Improved Stability: Increases a protein or peptide's resistance to denaturation and degradation [108].

**TABLE 5.3**
**Other Stealthing Agents**

| Sl No. | Material/Polymer/ Lipid Name | Nature | Functions | Reference |
|---|---|---|---|---|
| 1 | TPGS, or d-α-tocopheryl polyethylene glycol 1000 succinate | Nonionic surfactant with both hydrophobic and hydrophilic properties | Solubilizer, P-glycoprotein inhibitor, stabilizer, antioxidant, Enhances absorption | [93] |
| 2 | Poly (acryl amide) like N-(2-hydroxypropyl) methacrylamide (HPMA) | Nontoxic, highly water-absorbent, form a soft gel | Manufacturing stealth liposomes, thickener and suspending agent | [39] |
| 3 | Poly (2-methyl-2-oxazoline) | Thermosensitive materials | Extended blood circulation time and decreased uptake by the liver and spleen | [94] |
| 5 | Polyvinylpyrrolidone(PVP) | Water-soluble polymer | High stability and good processibility | [95] |
| 6 | Poly(hydroxyethyl l-glutamine) or Poly(hydroxyethyl-l-asparagine) | Poly(amino acids) | Potential stealth polymers, readily degraded by proteases and thus may reduce the risk of accumulation and related toxicity | [96] |
| 9 | Polysaccharides | Provide hydrophilic shells | Specific drug delivery to acidifying tumor along with extend circulation times | [37, 97] |
| 10 | Polyvinyl alcohol (PVA) | Synthetic, water-soluble polymer | Good biocompatibility, high water retention, good mechanical properties, and responsiveness to external stimuli | [98] |
| 11 | PLA (polylactic acid) | Biodegradable plastic made from lactic acid | Colloidal stabilizer | [99] |
| 13 | Dextran | Amphiphilic Polysaccharide | Stabilizer | [100] |
| 14 | Polyglycerols | Hydrophilic aliphatic polyether polyols | Anti-fouling effect of hyperbranched polyglycerols is comparable to PEG, less susceptible to oxidation or thermal stress than PEG | [101] |
| 15 | Poloxamer | Nonionic triblock copolymer | Stabilizer/emulsifier, poloxamer 188 onto nanoparticles can impart stealth property | [102] |
| 16 | Polyoxazolines | Amphiphilic block-co-polymer | Coupled with poly(caprolactone), poly(aspartic acid), or poly(1,3-trimethylene carbonate) to form polymeric micelles | [103] |

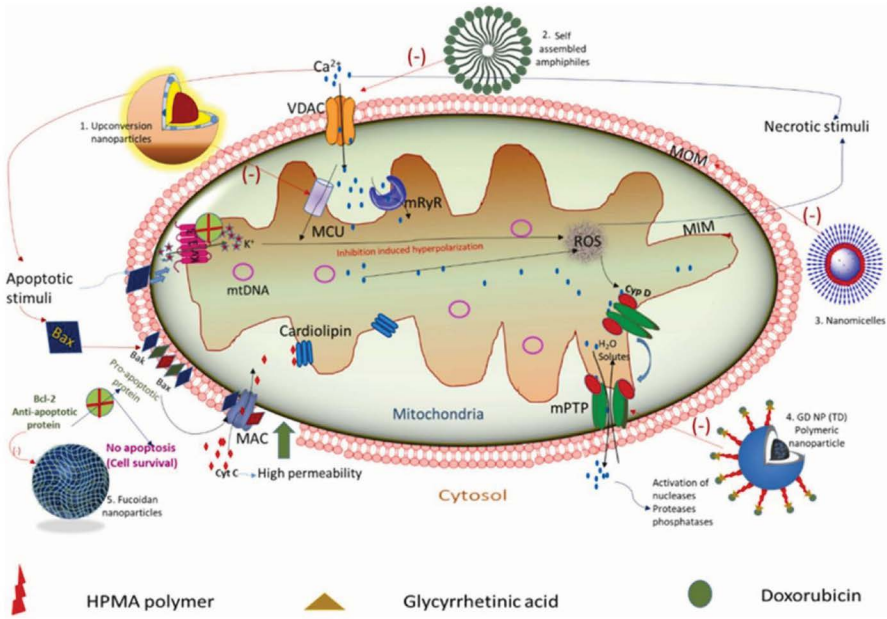



**FIGURE 5.5** Function of HPMA polymer. (Reproduced with the permission from Ref. [91].)

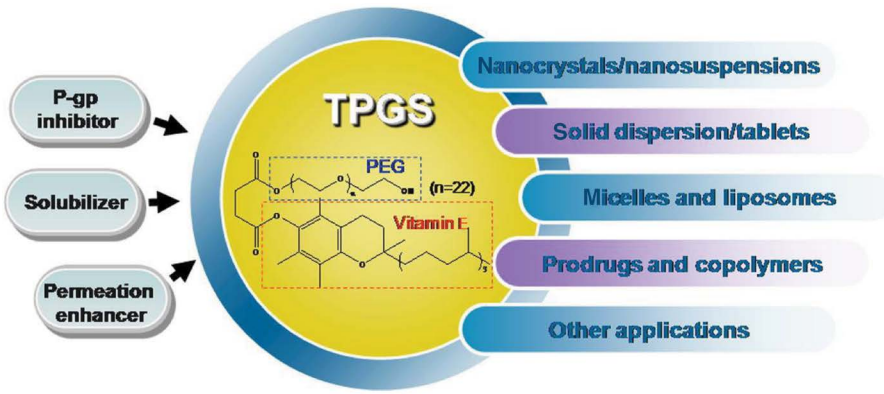



**FIGURE 5.6** Function of TPGS. (Reproduced with the permission from Ref. [93].)

## 5.5 LIMITATIONS/IMMUNOGENICITY OF PEGylated LIPOSOMES

PEGylated liposomes may be limited in a number of ways, including:

**Anti-PEG Immunity**: Anti-PEG antibodies may be produced as a result of repeated exposure to PEGylated formulations, which could speed up blood clearance and decrease the effectiveness of treatment. This is a serious issue, especially for long-term treatments [109].

**Complement Activation-Related Pseudoallergy (CARPA)**: As PEGylated liposomes, such as Doxil®, have been used increasingly often in recent years, metabolic responses have grown more common in clinical settings. Patients typically arrive with potentially fatal symptoms as flushing, rash, chest discomfort, facial edema, dyspnea, hypotension or hypertension, and heart pain. Up to 45% of patients treated directly with Doxil® without steroids and antihistamines experience allergic responses, while 4–7.1% of patients treated previously with steroids and antihistamines still experience them. The PEGylated liposomes in Doxil® are what trigger allergic reactions; doxorubicin by itself does not trigger these reactions. According to studies, complement activation and CARPA are closely associated. The more of the soluble non-functional form of the complement activation end product C5b-9, SC5b-9, there is, the more severe the reaction. In addition to inducing anti-PEGlgM by inducing an acquired immune response that activates complement via the classical pathway and causes severe CARPA, PEGylated liposomes can also initiate an innate immune response that activates complement. Furthermore, PEGylated liposomes have the ability to activate complement via other pathways, hence boosting the classical complement activation pathway through positive feedback. According to reports, patients who have experienced severe reactions to PEGylated liposomes have anti-PEGIgG titers that are roughly six times higher than those of non-reactive patients. Additionally, patients who have anti-PEG-IgG titers that are three times higher than the detection limit are roughly three times more likely to experience more severe allergic reactions than those whose titers are only one time higher [110].

**Aggregation**: PEG chain aggregation on the liposome surface can inhibit receptor-mediated endocytosis, which lowers medication uptake in cells. This is particularly pertinent to formulations that target particular cell receptors [111].

**Steric Hindrance**: The interaction of the liposome with target cells or tissues may be impacted by steric hindrance caused by the PEG coating. The density and length of the PEG chains determine this [112].

**Accelerated Blood Clearance (ABC) Phenomenon**: The ABC phenomenon in rats was found by Dams et al. in 2000. A second injection of PEGylated liposomes, spaced a few days apart from the first injection of either normal or PEGylated liposomes, caused a quick clearance and significant accumulation in the spleen and liver. A significant disadvantage of using liposomes in clinical settings would be this alteration in their pharmacokinetic characteristics. The buildup of liposomes in hepatic Kupffer cells could cause apoptosis and necrosis if the medicine enclosed in the liposome is cytotoxic. It would take two weeks for the Kupfer cells to recover, during which time cancer patients would die from bacteremia. The period between PEGylated liposome injections is one of the additional elements influencing the ABC phenomenon; the strongest ABC effect occurs at a seven-day gap. Furthermore, the intensity of the ABC phenomenon can be influenced

by the end groups, PEG density, and MW. The ABC phenomenon may also be impacted by the drug's dosage. For instance, when Doxil® was administered at a dose of 20 mg/m$^2$, no ABC phenomenon was shown in mice, *Macaca fascicularis*, rats, or minipigs; nevertheless, when 0.2 mg/m$^2$ was administered, ABC phenomenon was observed [113].

## 5.6 APPLICATIONS OF PEGylation

In therapeutics, it is widely used in biopharmaceuticals, including monoclonal antibodies, enzymes, and peptides (e.g., PEGylated interferon for hepatitis C). And also, in drug delivery systems of formulations like liposomes or NPs for cancer therapy, it helps improve bioavailability and targeting [8]. Several PEGylated drugs have been successfully developed and marketed, including PEGylated interferons (like peginterferon alfa-2a for hepatitis C) and PEGylated enzymes (such as pegaspargase for acute lymphoblastic leukemia). Similarly, in monoclonal antibodies, PEGylation is used to enhance the pharmacokinetics and efficacy of certain monoclonal antibodies. NPs and liposomes: PEGylated formulations are used in various drug delivery systems to improve solubility, stability, and targeting capabilities [114].

### 5.6.1 Scaling Up PEGylation

Manufacturing Techniques

- Continuous vs. Batch Processes: Continuous processes can be more consistent and efficient for commercial production, while batch processes are still often used, particularly for smaller-scale operations [115].
- Automated Systems: The use of automated systems helps standardize the PEGylation process, reducing variability and increasing reproducibility.

Purification and Characterization

- Separation Methods: To ensure high purity and activity, PEGylated compounds are purified using methods such as affinity chromatography, size exclusion chromatography, and ultrafiltration.
- Analytical Methods: Advanced characterization methods, such as mass spectrometry and NMR, are essential for confirming the degree of PEGylation and structural integrity [116].

## 5.7 CHALLENGES AND CONSIDERATIONS

- Complexity of Modification: Achieving consistent and reproducible PEGylation can be challenging, especially with site-specific methods [117].
- Biological Activity: The PEGylation process can sometimes impair the biological activity of the drug or protein, requiring careful optimization [1].
- Regulatory Issues: PEGylated products may face additional scrutiny in regulatory processes due to the complexity of the conjugation [118].

- Process Optimization: Achieving the right balance between PEGylation efficiency and retention of biological activity can be complex and may require extensive optimization [119].
- Immunogenicity: While PEGylation typically reduces immunogenicity, the introduction of PEG can still lead to immune responses in some patients, necessitating ongoing research into safer formulations [120].

## 5.8 REGULATORY CONSIDERATIONS

- Regulatory Pathways: PEGylated products must meet stringent regulatory requirements set by agencies like the FDA and EMA, which include demonstrating safety, efficacy, and quality through extensive clinical trials and manufacturing documentation.
- Post-Market Surveillance: Continuous monitoring is necessary to ensure long-term safety and effectiveness once PEGylated products are on the market [121].

## 5.9 FUTURE DIRECTIONS

- Innovative PEGylation Techniques: Research into novel PEGylation strategies, such as site-specific conjugation and using different PEG derivatives, continues to evolve.
- Biodegradable PEGs: The development of biodegradable PEG materials may enhance safety profiles and reduce environmental impact.
- Personalized Medicine: PEGylation techniques may be adapted for personalized therapies, tailoring PEGylated drugs to individual patient needs [122].

## 5.10 CONCLUSION

Commercial PEGylation is a proven technique that improves the safety and effectiveness of numerous biopharmaceuticals. In the production of biopharmaceuticals, PEGylation has emerged as a key approach, especially for enhancing the characteristics of therapeutic proteins and peptides. PEGylated medicines' uses and efficacy are expected to grow as a result of ongoing technological and regulatory developments. PEGylation is a strong and adaptable method that improves the pharmacological characteristics of medicinal substances. PEGylation techniques are being optimized through ongoing research to create better and more potent medications. Because of the severe issues with PEGylation, researchers have been searching for PEG substitutes like polyinosinic acid, polyglycidol, polyhydroxyethyl-L-asparagine, polysialic acid, and lipid derivatives containing sialic acid fragments. Unfortunately, no perfect substitutes for PEG have been found yet. In order to avoid intentionally seeking a "long acting," the relationship between liposomes and the immune system needs to be handled with caution. A crucial process in pharmaceuticals, PEGylation increases the therapeutic potential of drugs by improving their pharmacokinetics, stability, and solubility while reducing their toxicity and immunogenicity. This makes treatments more effective and more patient-friendly.

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# 6 Production of Lipid Nanoparticles for mRNA and Vaccines
## *An Industry Perspective*

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### 6.1 INTRODUCTION

Recent advances in mRNA design and delivery techniques have made mRNA vaccines a powerful tool against infectious diseases and cancer [1–3]. These vaccines offer several advantages over conventional vaccines, including improved safety, higher efficacy, greater potency, rapid and cost-effective manufacturing, and swift clinical development. mRNA plays a crucial role in translating DNA genetic sequences into proteins using ribosomes in the cell cytoplasm [1, 3]. There are two main types of mRNA used in vaccine research: non-replicating mRNA and self-amplifying mRNA [1]. Non-replicating mRNA-based vaccines encode the desired antigen for an immune response and include the 5′ and 3′ untranslated regions (UTRs), an open reading frame (ORF), and a poly(A) tail [1, 4]. Self-amplifying mRNA vaccines contain these components plus an additional coding region in the ORF that codes for viral replication machinery, enabling continuous intracellular RNA amplification and increased antigen expression [1, 5, 6]. Due to their immunostimulatory properties, mRNA vaccines can function as both adjuvants and immunogens. When administered intramuscularly, they can effectively activate the adaptive immune system [7].

The instability of mRNA in aqueous solutions poses significant challenges for its successful product development. To develop effective mRNA-encapsulated products, it is crucial to understand the factors responsible for mRNA degradation [8, 9]. Several factors can affect the stability of unencapsulated mRNA in aqueous solutions, including the integrity of the poly(A) tail and 5′ cap, RNA length, excipients (such as salts and buffering agents), the presence of enzymes like nucleases, divalent cations (e.g., $Ca^{2+}$ and $Mg^{2+}$), and the pH of the solution [1, 8, 9]. mRNA is highly sensitive to non-enzymatic degradation due to the presence of a 2′ hydroxyl group, which can cleave the backbone by attacking the adjacent phosphodiester bond ([0, 11]. Double-stranded nucleic acids are more unstable than single-stranded ones [10]. Stabilizing mRNA against physical

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and chemical degradation can enhance its biological function [10]. Physical instability includes aggregation, loss of stable structural conformation (secondary or tertiary structure), and precipitation, while chemical degradation occurs through hydrolysis and/or oxidation [9]. Chemical degradation can be enzymatically catalyzed by nucleases or non-enzymatically by water and factors like Bronsted acids, bases, or divalent cations [9]. mRNA can undergo acidic and alkaline hydrolysis of phosphodiester bonds, regardless of its structural conformation (single- or double-stranded). Reactive oxygen species are primarily responsible for mRNA oxidation [7, 9]. Chemical degradation plays a more significant role in mRNA degradation and reduced biological activity than physical instability, especially with a higher number of nucleotides. Additionally, mRNA stability is inversely related to its length [9, 12]. Encapsulating mRNA in suitable nanocarriers can mitigate some of these limitations by reducing its exposure to the external environment, thereby improving stability [12–14]. While naked mRNA, liposomes, and polyplexes have shown clinical effectiveness in humans, lipid nanoparticles (LNPs) for mRNA vaccines are the only drug delivery system that has demonstrated clinical effectiveness and received approval for human use [13, 14].

Moreover, literature indicates that mRNA vaccines exhibit similar and significant reactogenicity comparable to placebo groups. According to the Vaccine Adverse Event Reporting System (VAERS), the most frequently reported side effects associated with mRNA vaccines include fever, injection site pain, headache, nausea, neck pain, vomiting, diarrhea, drowsiness, dizziness, reduced alcohol tolerance, inflamed lymph nodes, dyspnea, stuffy nose, cough, fainting, excessive sweating, thirst, sore throat, insomnia, loss of appetite, irritability, photosensitivity, stupor, eye pain, malaise, and numbness in the extremities [15–17].

LNPs are a versatile class of nanocarriers widely used in medical research and pharmacology. They can encapsulate a variety of therapeutic agents, including nucleic acids, small molecules, and monoclonal antibodies, for diverse applications. Due to their structural similarity to cell membranes, LNPs can easily penetrate cell membranes [13, 18–21].

LNPs are primarily categorized into three types: (1) liposomes [22], (2) solid lipid nanoparticles (SLNs) [18], and (3) nanostructured lipid carriers (NLCs) [23].

- **Liposomes** are formed by the self-assembly of diacyl-chain phospholipids (lipid bilayers) in an aqueous phase. They are spherical and can be multilayered. Based on their size, structural complexity, and the number of lipid bilayers, liposomes are classified into four types: small unilamellar vesicles (SUVs), multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), and multivesicular vesicles (MVVs) [22, 24–27].
- **SLNs** are a pioneering class of lipid-based nanocarriers, made from lipids that are solid at body temperature and stabilized in an aqueous phase using suitable emulsifiers. These nanoparticles are submicron in size, typically less than 1,000 nm [18, 21, 28].
- **NLCs** represent the second generation of lipid nanocarrier systems, created from a mixture of liquid and solid lipids. NLCs are categorized into three types based on the drug incorporation method: Type I (imperfect type), Type II (multiple types), and Type III (amorphous type) [23, 29, 30].

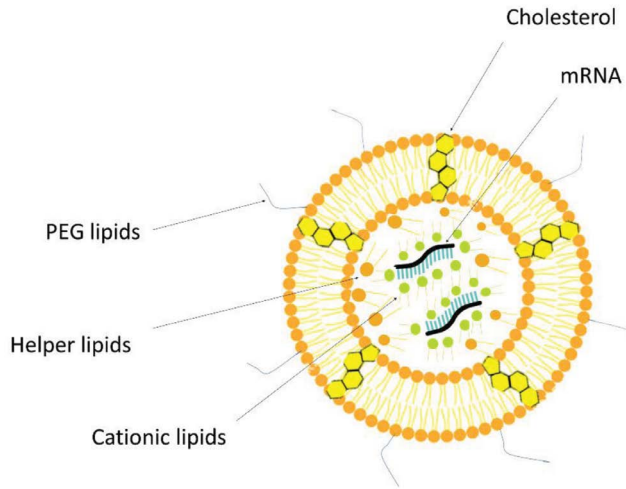



**FIGURE 6.1** Components of lipid nanoparticles (LNPs) [32].

The delivery of mRNA using LNPs is highly effective. LNPs facilitate the transport of mRNA to the cytosol. Figure 6.1 provides a graphical illustration of mRNA-loaded LNPs [31, 32]. The delivery mechanism involves the LNP attaching to the lipid bilayer of an endosome and releasing the mRNA into the cytosol, as shown in Figure 6.2 [32]. This process can be achieved using various delivery technologies, such as LNPs, polymeric nanoparticles [33], hybrid nanoparticles [34], and free mRNA solutions in appropriate buffers. However, LNPs are the most suitable option for mRNA delivery due to their biocompatibility and their ability to enhance the immune response to the mRNA [13]. Several types of carrier systems are used in lipid nanoparticulate systems, including liposomes, solid lipid nanoparticles, and NLCs [13, 31]. The main components of LNPs include cationic lipids, mRNA, helper

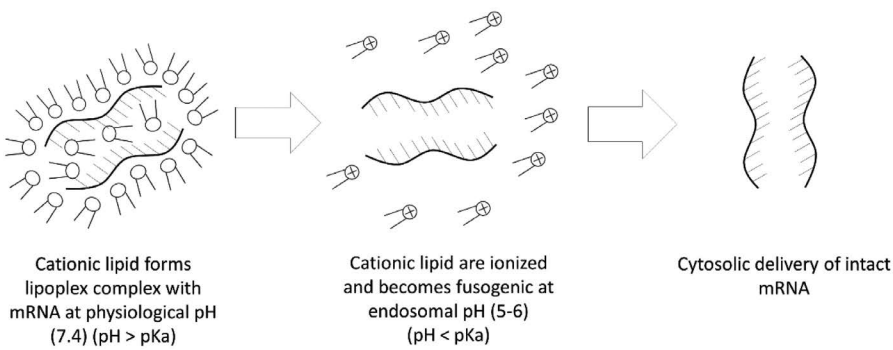



**FIGURE 6.2** Schematic representation of cytosolic release of mRNA from lipid nanoparticles (LNPs). pKa is a dissociation constant of cationic lipids [32].

lipids (such as phospholipids and PEG lipids), cholesterol, buffers, and modifying agents [13, 32].

Cationic lipids are crucial for mRNA delivery as they form complexes with mRNA, stabilizing it through electrostatic interactions due to the negative charge of nucleic acids. These lipids are vital for nucleic acid delivery and complexation, effectively entrapping mRNA and facilitating its release into the cytoplasm. To enhance their functionality, cationic lipids can be engineered with an appropriate acid dissociation constant (pKa) for improved endosomal release and complexation. At a pH lower than the pKa of the ionizable amino group, these lipids become protonated, generating a positive charge. Conversely, at a pH higher than the pKa, they remain neutral. This neutral state in the cytoplasm is key to delivering mRNA from LNPs while minimizing systemic toxicity. Examples of cationic lipids include: 1. Onpattro™: DLin-MC3-DMA, 2. Comirnaty™: ALC-0315, and 3. Spikevax™: SM102 [13, 32].

*Helper lipids:* These lipids are also known as structural lipids. There are two types of helper lipids: (a) PEG lipids and (b) phospholipids. A PEG lipid (pegylated lipids) mainly shield the LNP carrying the mRNA from any charge entities present during the delivery. This mainly helps LNPs by hindering their uptake via reticuloendothelial system (RES) and makes them circulate in the system. Additionally, PEG lipids prevent the protein binding of the nanoparticles and enhances its colloidal stability. This reduces RES-based clearance of a nanocarrier from the systemic circulation and thereby extending their circulation time. Preventing physical aggregation in solution of LNPs could enhance their storage stability which can be achieved by PEG lipid. Phospholipids mainly forms the lipid bilayer structure. Examples of this lipids are DSPC and DOPC. They also help LNP to bind with a plasma membrane/endosomal membrane and thereby releasing the content in cytosol [13, 32].

*Cholesterol:* As a neutral lipid, cholesterol increases the rigidity of the bilayer and stops the therapeutic ingredient from leaking, thereby improving bilayer stability. When utilized in the ideal concentration, it is also thought to contribute to gene transfer and membrane fusion for LNPs [13, 32].

There are certain factors which needs to consider while selecting cationic lipids like N/P ratio. Various LNP formulations were produced using a green fluorescent protein (EGFP)-labeled RNA which contain different cationic and helper lipids to obtain various N/P ratios, which ultimately determined a charge ratio and total mRNA-LNP net charge. A number of negative charges from the mRNA (one –ve charge per phosphodiester) and a number of positive charges from the lipid head group (one +ve charge per lipid head group) are generally used to calculate the overall charge ratio of the LNPs. The average molar mass of each nucleotide will be considered to be 330 Da to determine the molar ratio between the mRNA and cationic lipids. In general, mRNA is available in sodium citrate buffer (1 mm, pH 6.4) at a concentration of 1 mg/mL. The N/P ratio is obtained by diluting mRNA with desired buffer and then the suitable amount of LNPs is added to it to reach the desired charge ratio [13, 32, 35, 36].

Prior to 2018, no RNA vaccines had received approval. In 2018, Onapattro is an interfering RNA-based drug which is sold under the brand name **Patisiran.** It is approved by USFDA (US Food and Drug Administration) for the treatment of

polyneuropathy in people with hereditary transthyretin-mediated amyloidosis. It is a first iRNA-based molecule approved by the USFDA to manage this disease [37]. Then, a COVID-19 pandemic, triggered by the SARS-CoV-2 virus, brought mRNA vaccines into prominence. At that time, the USFDA granted emergency use approval for two mRNA-based vaccines: Pfizer–BioNTech on December 11, 2020, and Moderna on December 18, 2020. The Pfizer-BioNTech COVID-19 vaccine has been accessible under Emergency Use Authorization (EUA) for those who aged 16 and older since December 11, 2020. This authorization was extended to include those aged 12–15 on May 10, 2021. On August 23, 2021, USFDA granted full approval to the Pfizer-BioNTech COVID-19 vaccine which is now advertised as Comirnaty™ [38]. Similarly, Moderna has also submitted its COVID-19 vaccine for complete USFDA approval for those who are aged 18 and older. Apart from these approved products there is various ongoing clinical trials which are based on the mRNA-loaded LNP. These trials are listed in Table 6.1.

In this chapter we have discussed the scale of the mRNA-loaded LNPs. We had also discussed the common manufacturing technique for the preparation of LNP in brief. From these technologies, the technologies which can be used for the large-scale manufacturing are discussed here in detail.

**TABLE 6.1**
**Ongoing Clinical Trial of mRNA LNP (Obtained from Website: https://clinicaltrials.gov/)**

| Sr. No. | Disease | Phase |
|---|---|---|
| 1 | Cytomegalovirus (CMV) | Phase I |
| 2 | Rabies | Phase I |
| 3 | Tuberculosis | Phase II |
| 4 | hMPV and PIV3 | Phase I |
| 5 | Ovarian cancer | Phase I |
| 6 | Ornithine transcarbamylase deficiency | Phase I and II |
| 7 | Influenza | Phase I |
| | | Phase I |
| 8 | Cystic fibrosis | Phase I and II |
| 9 | COVID-19 | Phase I/II |
| | | Phase I |
| | | Phase I |
| 10 | Hereditary transthyretin amyloidosis with polyneuropathy | Phase I |
| 11 | Isolated methylmalonic academia | Phase I and II |
| 12 | Zika virus | Phase I |
| | | Phase I |
| 13 | Carnitine palmitoyl transferase 2 deficiency | Phase I |
| 14 | Triple negative breast cancer | Phase I |
| 15 | Propionic academia | Phase I and II |
| 16 | Carnitine palmitoyl transferase 2 deficiency | Phase I |
| 17 | Solid tumors | Phase I |

## 6.2 MANUFACTURING METHODS FOR DIFFERENT TYPES OF LIPID NANOPARTICLES

1. Liposomes
   i. *Thin-film hydration method:* Herein, lipids are first dissolved in an organic solvent. The solvent is then evaporated under vacuum at 45–60°C to form a thin film. This thin film is subsequently hydrated with an aqueous solution for 2 hours at 60–70°C to produce liposomal dispersion [39].
   ii. *Solvent injection methods:* Herein, lipids are first dissolved in organic solvent and injected into aqueous medium using appropriate needle with continuous stirring to prepare homogenous liposomal dispersion. Then, the prepared liposomal dispersion must be heated to remove an organic solvent [40].
   iii. *Detergent removal method:* This technique includes the hydration of lipids using detergent solution. During hydration, a detergent will react with phospholipids and forms mixed micelles. On successive removal of detergent from solution, mixed micelles increase in the lipids and eventually forms unilamellar liposomes [41].
   iv. *Reverse-phase evaporation method:* First, in a mixture of the organic phase, lipids are dissolved, forming reverse micelles. Upon the addition of an aqueous medium, at the interface between oil and water, the lipids rearrange themselves, resulting in a water-in-oil (W/O) microemulsion. The prepared microemulsion will then be subjected to slow solvent evaporation under vacuum until a viscous gel form. Subsequently, the organic solvent will be slowly removed to disrupt the inverted micelles, ultimately promoting the formation of liposomes [42].
   v. *Downsizing and post-formation processing:* After the preparation MLVs, its size can be reduced to desired level using three approaches:

      Membrane extrusion method: In this method, a polycarbonate membrane filter with a fixed pore size is used to reduce the particle size of the liposomes by passing them through the filter at low pressure. The liposomal dispersion is passed several times through the polycarbonate membrane till desired particle size is achieved. While passing through this membrane, breaking and resealing of phospholipids occur [39].

      High shear homogenization: In this process, the liposomes are uninterruptedly passed through the orifice at very high pressure. Because of this, liposomes collide with the stainless-steel wall of the homogenizer and produces reduce size liposomes [43].

      Sonication: This technique is extensively use for small unilamellar liposome preparation. Mainly, two sonication technique can be used for the preparation of liposomes: probe sonication and bath sonication [44]
   vi. *Microfluidic channel method:* Lipids are first dissolved in an organic solvent (such as ethanol, methanol, butanol, or isopropyl alcohol) in this technique. They are then passed through the center of a channel filled with aqueous media. During this process, the lipids from the stream mix with the aqueous media, forming liposomes [45].

vii. *Heating method:* In this method, phospholipids are hydrated with polyethylene glycol/ethylene glycol or glycerol while continuously stirring for 1 hour at 60 or 120°C. Then, the formed mixture will be cooled and centrifuged to obtain or separate the prepared liposomes [46].

viii. *Freeze drying method:* The lipids, along with the drug and lyoprotectant, are dissolved in tert-butyl alcohol and water, respectively, at a temperature of 45°C. Once dissolved, they are mixed together to form a monophasic solution. This solution is then filtered and subjected to freeze drying to harvest proliposomes. The freeze-drying process consists of two steps: first, the sample is frozen at –40°C, and second, it is dried at room temperature. Upon hydration, this results in the formation of liposomes [46].

ix. *Supercritical anti-solvent method (SAS):* This technique is widely popular for preparing proliposomes due to its numerous advantages, including simpler manufacturing, low solvent residue, and suitability for drugs with low solubility in supercritical fluids (SCFs) used as anti-solvents. In this method, an SCF is passed through the top of a vessel under high pressure. A drug solution is then atomized into small droplets using an atomizing nozzle on this SCF solvent. Liposomes are formed by hydrating these droplets with an aqueous solution. A common SCF used in this process is CO2, which acts as an anti-solvent to the solute but is miscible with organic solvents. Compared to conventional methods, liposomes prepared using this technique are free from toxic organic solvents. An advanced version of this method is known as the "Continuous Anti-Solvent (CAS)" method [46].

2. NLCs and SLNs

i. *High-pressure homogenization:* In this technique, coarse lipid particles are first prepared. These particles are then passed through a homogenizer with a narrow gap of a few microns under high pressure. A high pressure accelerates the particle dispersion to the velocity of >1,000 km/h, so that particles experience high shear stress and cavitation forces and reduces to nano scale. The homogenization is continued till the required particles size and uniformity is attained. There are two types of homogenization which can be used to prepare SLN and NLC: cold and hot homogenization [47, 48].

ii. *High-speed stirring and ultra-sonication:* The lipids are initially melted at a temperature typically 5–10°C above their melting point. Then, in this molten mass, drug to be entrapped is dispersed or dissolved. An aqueous solution comprising suitable stabilizer or surfactant is added to lipid-drug mixture with continuous stirring using high-speed stirrer till homogenous hot oil/water emulsion is resulted. An SLN and NLC can be obtained on cooling this emulsion. After high-speed stirring, an ultra-sonication can further reduce the droplet size. Thus, a high-speed stirring and ultra-sonication are usually employed in combined technique to attain desired particles size having narrow particle size distribution range [49].

iii. *Microemulsion method:* In brief, this technique involves dissolving or dispersing a drug in molten lipids. An aqueous solution containing water and surfactant is then added under mild stirring to form a microemulsion. This microemulsion is subsequently added to a cold aqueous solution (25–50 times the volume) under gentle stirring. Upon dilution, the lipids recrystallize from the mixture, forming SLNs and NLCs [50, 51].

iv. *Solvent emulsification-diffusion method:* In this method, organic solvents that are partially miscible with water are used. First, a saturated solution of water and organic solvent is prepared, in which lipids and drugs are dissolved. This solution is then emulsified with an aqueous phase under stirring to form an oil/water emulsion. The emulsion is further diluted using water to permit the organic solvent to diffuse into the continuous phase. This causes the lipids to precipitate, forming NLCs and SLNs. Finally, a removal of an organic solvent can be done via lyophilization or vacuum distillation [49, 52].

v. *Solvent emulsification-evaporation method:* This technique utilizes the water-immiscible organic solvent rather than water-miscible solvent. First, the lipids and drugs are dissolved in the organic solvent and then it is emulsified in aqueous phase to prepare oil/water emulsion. Then, this emulsion is subjected to the solvent evaporation using mechanical stirring or rotary evaporator to remove the organic solvent, which eventually lead to the formation of SLNs and NLCs due to recrystallization of lipids [18].

vi. *Double emulsification method:* A surfactant and a drug are dissolved in aqueous phase according to this method. Then this surfactant and drug solution is emulsified in organic solvent having lipids or molten lipids which results into water/oil emulsion. This organic solvent must be immiscible with water. Then, this prepared emulsion is dispersed in surfactant containing aqueous medium to form water/oil/water multiple emulsion. On organic solvent evaporation, lipids get reprecipitated and form SLNs and NLCs [53].

vii. *Phase inversion temperature method:* In this technique, temperature-based phase inversion (PIT) of emulsion occurs like water/oil to oil/water or vice versa. A non-ionic surfactant having temperature-dependent properties are required to prepare NLC or SLN by this technique. These surfactants have high HLB value at low temperature which means high hydration of its hydrophilic groups. However, as temperature increases, dehydration of this hydrophilic group takes place and lipophilicity of surfactant increases and HLB value decreases. A PIT is a temperature at when surfactants have an equal affinity toward the aqueous and lipid phases. Thus, when temperature is greater than PIT, w/o emulsion is formed because of the higher lipophilicity of a surfactant and when temperature less than PIT, it forms o/w emulsion. So, now to prepare SLNs and NLCs based on this principle, first surfactant is heated at a temperature greater than PIT which forms w/o emulsion. Then, it is cooled rapidly with constant stirring which leads to the break down of a w/o emulsion and forms o/w emulsion. During

this SLNs or NLCs are produced due to reprecipitation of lipids at low temperature [54].

viii. *Membrane contactor method:* While keeping a temperature of lipid phase more than lipid's melting point, it is passed through the membrane's pores. This results in formation of small droplets of lipids which are cooled by aqueous phase containing surfactant on the other side. An aqueous phase will be maintained in continuous motion so that it can sweep away the lipids droplets as it forms at pore outlets [55].

ix. *Supercritical fluid-based methods:* This method employs supercritical fluid (SCF), such as supercritical $CO_2$. In the supercritical fluid extraction of emulsion (SFEE) process, an oil-in-water emulsion is first formed, followed by the extraction of the organic solvent using SCF. Typically, the emulsion is added at the top of the extraction column, while the SCF is introduced from the bottom in a counter-current manner. This process rapidly and completely removes the solvent, resulting in the formation of SLNs or NLCs due to lipid precipitation [56].

x. *Coacervation method:* This method utilizes a principle of an acidification-based precipitation of an alkali salt of fatty acids. An alkali salt of lipids is dispersed homogenously in polymeric stabilizer solution. A solution is then heated above lipid's kraft point with continuous stirring till clear solution is obtained. After obtaining clear solution, a drug dissolved in ethanol is added into it and stirred till single phase solution is obtained. Then, a coacervating solution or acidifying solution is added into it dropwise with constant stirring till precipitation of lipids starts, which ultimately results into formation of NLCs or SLNs [18].

xi. *Solvent injection method:* A drug and lipids are first dissolved in organic solvent which is water miscible based on this method. Then, aqueous phase is prepared by dissolving suitable emulsifier in a water. Then, a lipidic phase is added in the aqueous phase dropwise using hypodermic needle with continuous mechanical agitation. On adding in aqueous phase, solvent from lipidic phase is diffused in an aqueous phase which results into precipitation of lipids and forms SLNs or NLCs [18, 56].

Above-mentioned methods are common techniques which can be used to prepare LNPs. However, every above-mentioned technique cannot be used for the manufacturing of mRNA-loaded LNPs due to the complexity of handling of mRNA as mentioned above. For that very few techniques are available like ethanol injection method, microfluidizer, etc. Much elaborated research work is needed to comprehend the feasibility of other methods for the preparation of mRNA LNP.

## 6.3 SCALE-UP OF MANUFACTURING PROCESS FROM LABORATORY SCALE TO COMMERCIAL SCALE

Considering that most LNPs formulations aim mainly to reduce the toxicity and enhance drug targeting an entrapped drug, a preparation method must meticulously regulate the LNPs critical quality attributes (CQAs). These attributes encompass high drug loading

and retention, particle size, and PEGylation and/or surface charge [57]. While many techniques are there for preparing LNPs at the lab scale, only selected few are suitable for large-scale manufacturing that meets the necessary CQAs. Among these techniques, ethanol injection followed by extrusion is a furthermost common technique employed for commercial LNPs production. This method is preferred due to its reproducibility in achieving consistent LNPs polydispersity index (PDI) and particle size, as well as the advantage of employing solvent diffusion over solvent evaporation [58].

A commercial manufacturing of LNPs is a lengthy and labor-intensive method, involving numerous unit operations and exhaustive analysis. A general process includes buffer preparation, filtration, phospholipid solution preparation, lipid hydration, extrusion, diafiltration, dilution, sterile filtration, and filling [59]. Each step requires in-process quality control, adding to the overall complexity. In-process quality control involves pH measurement at each critical step, phospholipid content analysis, filter integrity tests, bulk drug product assays, zeta potential and particle size measurements, visual inspection and bioburden testing [59]. This basic process does not account for additional complexities such as active loading or freeze-drying, which further complicate the process [59]. For instance, a large-scale LNPs manufacturing process via an ethanol injection method followed by extrusion for a hydrophilic and hydrophobic drug encompasses around 9-unit operations as described in Figure 6.3. In-process quality control is mandatory at each unit operation, making the process long and labor-intensive [59, 60].

A process of scaling up of manufacturing procedure from a lab scale to large-scale has its own drawbacks which requires to be addressed for successful transfer of process. There are several factors which can affect the scaling up process like energy transfer rate, mass transfer rate, batch size/volume etc. In general, scale-up of laboratory level process to commercial level undergoes three main phases. The first and initial stage of this process is laboratory scale work. This stage is required to obtain the necessary information regarding the manufacturing process like mass transfer, reaction kinetics of process, fluid hydrodynamics of process, etc. This data is crucial for the research scientist to understand the reaction mechanism of species and product formation, enabling effective optimization. Then comes the second stage which is pilot plant batch level. During this stage, laboratory-scale parameters are assessed in the pilot plant to optimize the process before transitioning to larger commercial production—last third phase. However, altering the batch volume during different phases fundamentally introduces complications. These changes affect heat and mass transport behavior, ultimately impacting the quality of a finished product in commercial processes [58, 61–63].

In addition to the complexities of large-scale LNPs manufacturing, there are several CQAs of LNPs that require vigilant attention. These attributes can impact the final product during manufacturing, storage, or clinical performance [58, 61–64]. The CQAs are outlined below:

1. Appearance: A free and entrapped drug, both can influence the appearance of the LNP product. Other attributes such as zeta potential and particle size can also affect the product's appearance. Apart from these, appearance is also made release criterion by Regulatory guidelines [65].

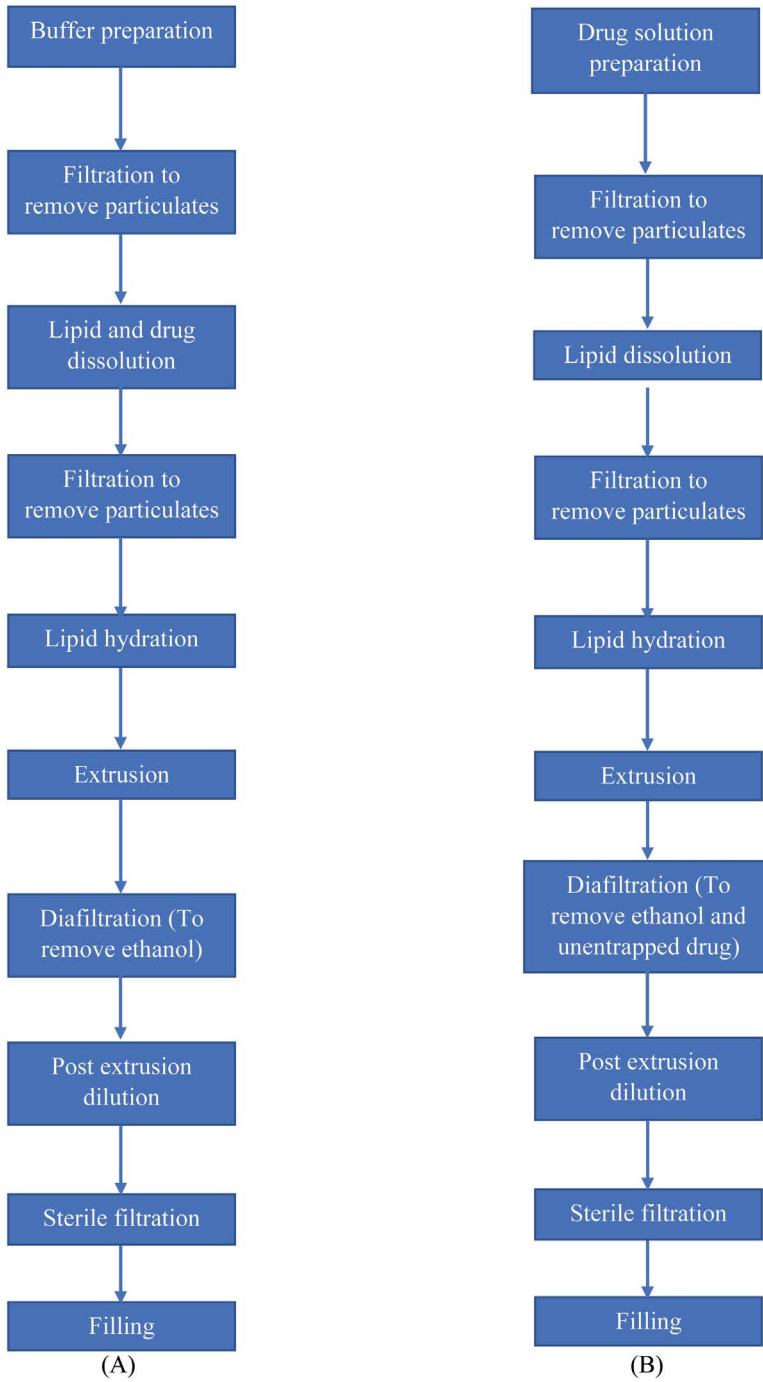



**FIGURE 6.3** Manufacturing steps for ethanol injection method: (A) for hydrophilic drug and (B) for hydrophobic drug.

2. Identification of drug: Ensuring patient safety necessitates the identification of the drug. Administering an incorrect drug can be hazardous to the patient. Thus, it is made a release requirement by the Regulatory guidelines. Notably, any process parameters can't influence the identification of the drug [65].
3. Identification of phospholipids: A major building block for any LNP is phospholipid. Hence, it is important for LNP preparation. An incorrect phospholipid during manufacturing of the LNP would results into the product which has a deformed structure or maybe the LNP particles which do not have desired characteristics [65].
4. Identification of cholesterol: Cholesterol provides rigidity to the LNP structure and hence prevents leakage from it. Thus, cholesterol is very important for the performance of LNPs *in vivo* [65].
5. Assay of drug: Suitable drug plasma level is crucial for effectively engaging the pharmacological target during treatment. Administering a lower dose may result in insufficient effectiveness, with the impact on the patient depending on the duration without proper medication. Conversely, a higher dose could compromise patient safety. Regulatory guidelines also mandate assay analysis for release criterion [65–67].
6. Percent drug encapsulation: In certain cases, free drug (unentrapped drug) can exhibit toxic or adverse reactions *in vivo*. Therefore, determination of the entrapment efficiency of the drug is crucial for any LNP system. Moreover, measuring the amount of free drug is also a regulatory requirement. A complete or near to 100% entrapped drug will be responsible for the sustained release of a drug. However, for the mRNA, a free drug will be deactivated by the enzymes present in the body. Thus, complete or near to 100% entrapment is require for the mRNA-loaded LNP [65–67].
7. Particle size diameter and PDI: A mean particle diameter significantly influences the circulation properties of LNPs, which in turn impact patient safety. Additionally, a particle size affects the release kinetics of a drug from the LNPs, ultimately influencing the efficacy of the final product. The PDI is a crucial parameter for characterizing LNPs. It measures the uniformity of particle sizes within a sample. For LNPs, a uniform particles size is indicated by the lower PDI, which is required for reliable performance and stability. Typically, narrow size distribution is indicated by the PDI value between 0.1 and 0.25 which means uniformity. While a broad size distribution is suggested by the PDI value above 0.5, which can affect the consistency and performance of the nanoparticles. The PDI is essential for assessing the quality of LNPs, as it impacts their safety, stability, and efficacy. A lower PDI is generally preferred for drug delivery application and other biomedical uses. Therefore, regulatory guidelines mandate the measurement of particle size and PDI [65–67].
8. Content uniformity: A therapeutic dose that a patient is getting and thus the efficacy of a product can be affected by the content uniformity of the product. A high dose may result into toxicities while low dose is responsible for the less effectiveness of a product.

9. pH: The pH level affects the stability of both drug and lipids. An unstable lipid can alter the release of an mRNA from the LNPs. Moreover, any deviations in pH can also cause injection site irritation and results into poor patient compliance [65–67].
10. Extractable volume: The vial should be filled with a volume that allows for the complete withdrawal of a unit dose in a clinical setting. So that sufficient dose can be delivered to the patient [65–67].
11. Osmolality: Different osmolality can result into injection site irritation. Apart from this, osmolarity also impacts the stability of the prepared LNP. In case of liposomes, if the external environment is isotonic or hypertonic, it will disturb the drug release kinetic from the liposomes and inversely affect the effectiveness of the product [65–67].
12. *In vitro* drug release: For ensuring an *in vivo* release kinetic of a product and to verify the absence of unrestrained leakages of the drug in various physiological conditions, an *in vitro* drug release testing is performed. Results should fall within the acceptable criteria [65–67].
13. Residual solvents: Most commonly organic solvents are used for the preparation of the LNP. However, to obtain the optimal performance of a nanocarrier and also to improve patient safety profile, these organic solvents need to be removed. Residual solvents can also result into the drug leakage from the LNPs. Moreover, residual solvent can also cause some toxic effects to the patients [65–67].
14. Rabbit pyrogen test or LAL test: Pyrogens can induce anaphylactic reaction in patients if delivered parenterally. So, pyrogen detection test like rabbit pyrogen test and LAL (limulus lysate test) test needs to be performed for the finished as well as in process sample. Thus, it is mandatory to control it below limit [65–67].
15. Sterility: A microbial contamination in an injection product can lead to serious infections. Thus, this test is mandatory for regulatory perspective [65–67].
16. Zeta potential: The zeta potential of LNPs measures an electric potential at the interface between a particle surface and surrounding liquid. This potential is essential for understanding the stability and behavior of nanoparticles in suspension. In drug delivery applications, such as mRNA encapsulation, the zeta potential can affect the efficiency of cellular uptake and the release of the therapeutic payload. Ionizable lipids, which alter their charge with pH, are often utilized to optimize the zeta potential for improved delivery and reduced toxicity [65–67].

## 6.4 COMMERCIAL MANUFACTURING DEVELOPMENT BASED ON QbD APPROACH

The development of new formulation by traditional way is very long, expensive, and difficult process. However, this approach ensures quality of product by testing instead of by manufacturing which is quite expensive for both development and manufacturing perspective [68, 69]. Thus, to ensure product quality and reduce development cost,

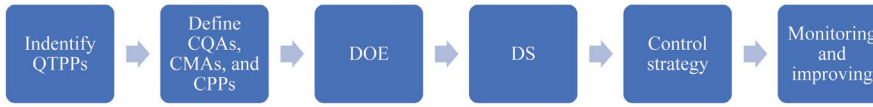



**FIGURE 6.4** Schematic flow diagram of QbD for a formulation development.

Quality by Design (QbD) approach was introduced. QbD is a structured approach to drug and drug product development. It relies on predefined criteria, a deep knowledge of both the process and the product, and thorough quality risk assessments [68, 70]. A general flow chart using QbD is represented in Figure 6.4. A QbD approach for product development can be utilized by the following steps: (1) Define main Quality Target Product Profile (QTPP), (2) Define CQAs, Critical Material Attributes (CMAs), Critical Process Parameters (CPPs) which can impact product quality, (3) Appropriate Design of Experiment (DoE) is used to identify the Design Space (DS), (4) To define DS which can give final product with targeted QTPP, (5) To create a risk control strategy aimed at identifying the sources of variability, and (6) Ongoing monitoring and enhancement of a manufacturing process [68–70].

### 6.4.1 Quality Target Product Profile (QTPP)

A QTPP outlines the attributes and characteristics that determine a product's quality. It includes quantitative data supporting clinical safety and efficacy, forming the basis for designing and optimizing formulations or manufacturing processes. The USFDA emphasizes that QTPP focuses on the chemical, manufacturing, and control stages of development. It specifies the drug's quantitative characteristics, such as application, dosage form, appearance, packaging, and diagnosis. Examples of QTPP elements include the route of drug administration, clinical use, dosage form, drug delivery system, therapeutic dose, factors affecting pharmacokinetic parameters, packaging, and quality criteria of the finished product like drug release, sterility, and stability during storage. Generally, QTPP encompasses only the product properties relevant to patients, which help us to optimize process parameters to make sure that CQAs are within a predefined range [64, 68–72].

### 6.4.2 Critical Quality Attributes (CQAs)

CQAs are the physical, chemical, microbiological, or biological properties that must be measured to guarantee the quality of a product. These attributes influence the QTPP and are essential for maintaining product quality. Typically, CQAs are associated with the appropriate selection and quantities of APIs (active pharmaceutical ingredients) and excipient [68–72].

### 6.4.3 Critical Process Parameters (CPPs)

CPPs are production attributes that influence CQAs and must be precisely measured. For instance, attributes that impact the finished product quality must be supervised

throughout the manufacturing cycle. An attribute is calculated or measured property of a process or system. These parameters are the part of a production cycle and typically include characteristics of processes or materials that impact manufacturing, such as stirring speed, temperature, flow rate of different phase solution prepared, and composition [68–72].

### 6.4.4 Critical Material Attributes (CMAs)

CMAs are the characteristics of ingredients that must meet specific boundaries to ensure the quality of drugs, excipients, and other ingredients employ in manufacturing cycle. It's crucial to distinguish between CQAs (output data) and CMAs (input data) during development. For instance, the CQAs of an intermediate product may become the CMAs for the next production step. According to the QbD approach, CMAs and CPPs can vary within the defined DS without significantly affecting CQAs. Additionally, following the ICH guideline "Quality Risk Management" (ICH Q9), identifying potential causes of process variability and conducting effective risk analysis are key during process optimization. This procedure can be performed at any stage, with the possibility of repetition or redefinition as needed. Thus, the quality of the final product is ensured through prior experience in determining CQAs, CPPs, and conducting risk assessments [68–72].

### 6.4.5 DoE

Once the CQAs and CPPs are finalized, DOE is employed to optimize the formulation. For this optimization, an experimental matrix model is chosen from various available DOE models, such as 2-factor design, 3-factor design, central composite design, Box-Behnken design, and fractional factorial design. After selecting the design, a series of experiments is suggested, which are then conducted. The results are fed into the software, which subsequently provides the DS [68–72].

### 6.4.6 Risk Assessment

Risk assessment helps identify potential issues, their likelihood, and their impact on product quality. Risk control involves procedures to mitigate these risks based on the ICH Q9. Common risk management tools are included in this guideline. In this step, it's crucial to determine which risks are acceptable on other hand which need to be controlled. A multiple regression analysis and ANOVA (Analysis of Variance) are most common tools use for the risk management based on the data obtained during an experimentation. Multiple regression analysis can derive a mathematical formula showing a connection between the attributes, while ANOVA assesses the mathematical important of every variable and their interaction impacts. A risk assessment strategy should be used to monitor the results, considering prior experience and knowledge in the last stage. QTPPs, CPPs, and CQAs can be identified based on the prior knowledge and experience. This can be done using numerous risk assessment tools like failure mode and effects analysis, risk filtering, and Ishikawa diagrams [68–72].

### 6.4.7 Design Space (DS)

A DS can play a crucial part in the pharmaceutical world, defining the multivariate functional relationships between CQAs and CPPs. These relationships are identified through DoE, modeling, and risk assessment [68–72].

The DS is explicit to a single manufacturing procedure or a unit operation, outlining operating process factors which can impact product quality. It serves as a link between CPPs and CQAs, illustrating the development of process understanding. The advantages of creating a DS are clear, though the cost of its creation can be a challenge [68–72].

A key aspect of developing the DS is demonstrating that uncategorized variables omitted from the DOE are non-critical. Thus, they do not interact with each other. DOE screening helps identify interactions between process parameters. If no interaction is found, single-variable intervals can be added to the DS as non-critical parameters [68–72].

### 6.4.8 Control Strategy

A product quality and process performance can be easily managed by using a control strategy. A control strategy is carefully prepared based on the detailed process and product knowledge. A control strategy is prepared with the formulation development offers the deep understanding for process and product according to the QbD approach. QbD-based control strategies provide more comprehensive information, making them beneficial for areas demanding extensive time and specialized information compared to standard methods. This novel method for ensuring product quality focuses on establishing the final product's quality by pinpointing and managing an ideal range of product and process attributes, followed by final testing to verify the product's quality [68–72].

A precise effective control strategy can be developed by risk-based approach to achieve the final product's QTPP. It starts with the QTPP. Initial studies focus on characterizing a drug and key chemical, physical, microbiological, and biological characteristics of a formulation. During this stage, a process development should also be cleared. Let's assume, if a drug has less aqueous solubility, than an adequate release of drug can be safeguarded by converting it into immediate-release tablet. An impurity profile of a drug can be prepared by performing initial toxicity studies. To prepare the product specification criteria, identification of steps for control strategy, knowledge of impurities removal and generation, and developing acceptance criteria are necessary [68–72].

The quality risk management principles outlined in the ICH Q9 guidelines can also be applied to develop an appropriate control strategy beyond the steps already mentioned. Stabilizing a process requires a thorough understanding and continuous control strategy during production. It's crucial to implement a QbD approach within the pharmaceutical quality system. In commercial production, the control strategy should be adaptable to accommodate updates, modifications, and continuous improvements. When updates to operating procedures, documents and product information, the pharmaceutical quality system must conduct the essential administrative and technical audits to secure the necessary approvals and reviews [68–72].

## 6.5 QbD AND DOE FOR THE DEVELOPMENT OF mRNA-LOADED LNP

A development of an mRNA-loaded LNP using QbD approach is thoroughly linked to its structure and pharmaceutical goal [64, 73–75]. The following parameters can be scrutinized for the development of mRNA-loaded LNP based on the various published literature:

*QTPP:* safety and efficacy [64, 73–75]

*CMAs:* stabilizer type, N/P ratio, lipid type, biodegradability, non-toxicity, etc. Various types of lipids are used for the development of an mRNA LNP [64, 73–75]. Some of them are listed in Table 6.2.

*CQAs:* particle size, polydispersity index (PDI), % encapsulation, zeta potential, stability, % drug loading, lipids contents, shelf life, storage condition, etc. [64, 73–75]

*CPPs:* temperature, flow rate, filtration, stirring speed, microfluidics, etc. [64, 73–75]

## 6.6 COMMERCIAL MANUFACTURING METHOD

1. Direct dilution method
   An LNP is manufactured using this method as per following steps (Figure 6.5):
   i. Preparation of lipid and active therapeutic ingredient (API) solution
      a. *Lipid solution:* A lipid solution is prepared by dissolving various lipids in suitable organic solvent using an overhead stirrer at a suitable temperature. A different kind of lipids which can be used to prepare LNPs are: phospholipid, cholesterol, cationic lipid, PEG-lipid, etc. These lipids are dissolved in organic solvent in different proportion based on a type of lipid particles is manufactured. Any of the following organic solvent can be used to prepare a lipid phase: methanol, ethanol, propanol, butanol, pentanol, and their isomers. They can be used in combination also [101].
      b. *API solution:* An API solution is prepared by dissolving API in suitable buffer having required pH range. Here, API can be any nucleic acid-based therapeutic molecules like antisense DNA, plasmid DNA, pre-condensed DNA, vectors, chromosomal DNA, tRNS (transfer RNA), mRNA (messenger RNA), snRNA (small nuclear RNA), siRNA (small interfering RNA), oligonucleotide RNA, rRNA (ribosomal RNA), shRNS (short hairpin RNA), ncRNA (non-coding RNA), ribozymes, aptamers, etc. [101]
   ii. LNP formation: After the preparation of these two solutions, they are mixed together using peristatic pump or pulseless gear pump. To prepare the LNPs, these solutions are pumped at equal flow rate in opposite direction in the mixing environment. In some cases, non-equal flow rate can also be used. A mixing area can be a mixing chamber or T-shaped connector. For, T-shaped connector it is ideal that a fluid line meets in a narrow aperture in T-shaped connector as the opposite flow.

**TABLE 6.2**
**Types of Lipids Used for the Preparation of mRNA LNP**

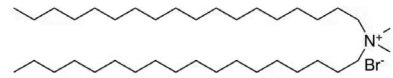
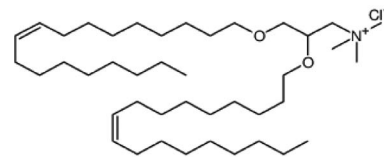
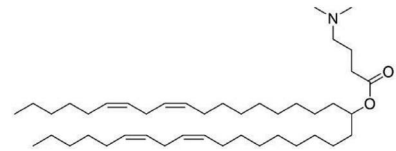
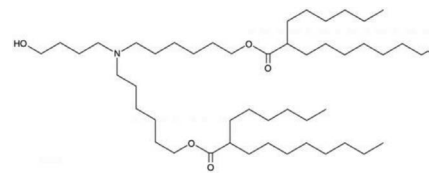
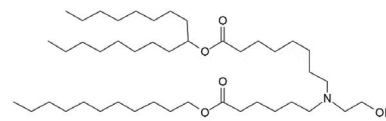
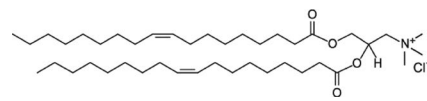
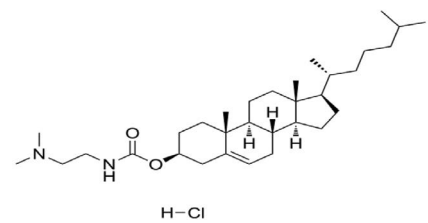
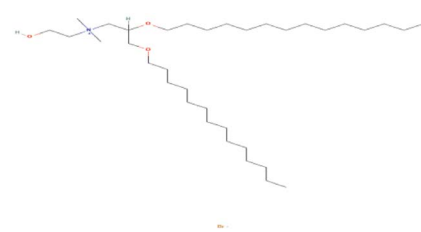
| Sr No | Name of Lipid | Role | Structure |
|---|---|---|---|
| 1 | Didodecyldimethylammonium bromide (DDAB) [76] | Cationic lipid |  |
| 2 | N-(1-(2,3-Dioleyloxy) propyl)-N,N,N-trimethylammonium chloride (DOTMA) [77] | Cationic lipid |  |
| 3 | DLin-MC3-DMA [78] | Cationic lipid |  |
| 4 | ALC-0315 [79] | Cationic lipid |  |
| 5 | SM102 [80] | Cationic lipid |  |
| 6 | Dioleoyl-3-trimethylammonium propane (DOTAP) [81] | |  |
| 7 | Cholesterol (2-dimethylaminoethyl) carbamate (DC-Chol) [82] | Cationic lipid |  |
| 8 | (3-dimyristyloxypropyl) (dimethyl) (hydroxyethyl) ammonium DMRIE [83] | Cationic lipid |  |

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**TABLE 6.2** ***(Continued)***
**Types of Lipids Used for the Preparation of mRNA LNP**

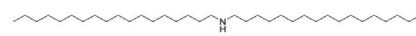
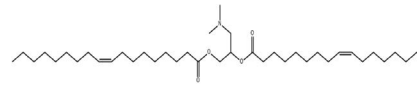
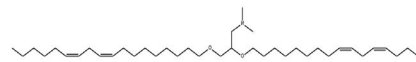
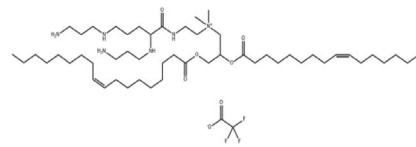
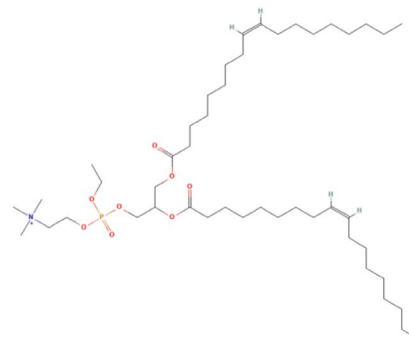
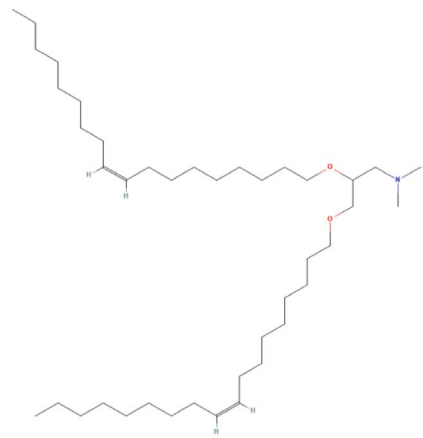
| Sr No | Name of Lipid | Role | Structure |
|---|---|---|---|
| 9 | Dioctadecylamine [84] | Cationic lipid |  |
| 10 | DODAP (1,2-Di(oleoyloxy)-3-(dimethylamino) propane) [85] | Cationic lipid |  |
| 11 | DLinDMA [86] | Cationic lipid |  |
| 12 | DOSPA [87] | Cationic lipid |  |
| 13 | EDOPC [88] | Cationic lipid |  |
| 14 | 1,2-dioleyloxy-3-dimethylamino-propane (DODMA) [89] | Cationic lipid |  |

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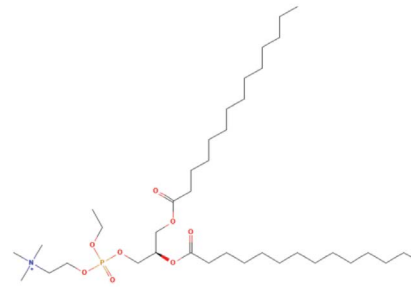
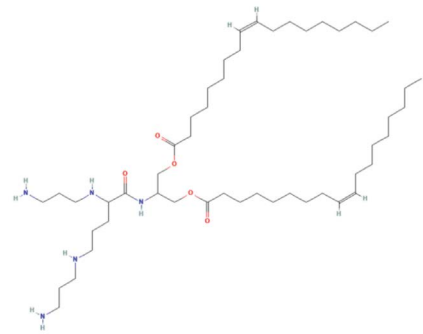
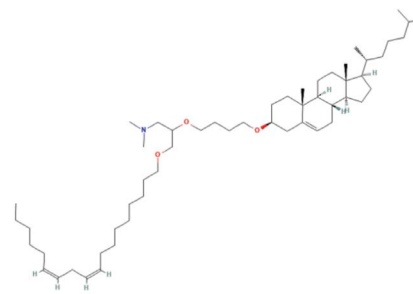
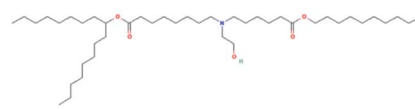
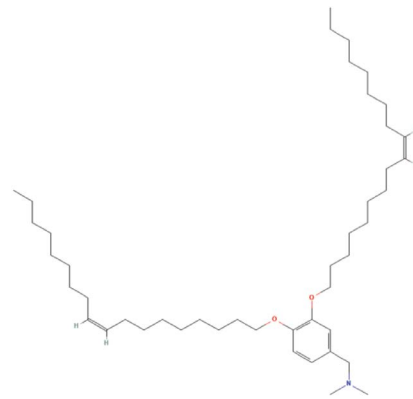
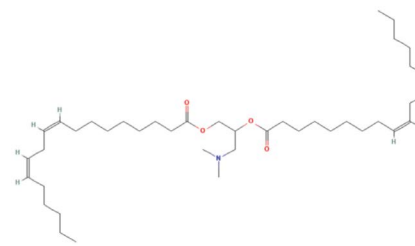
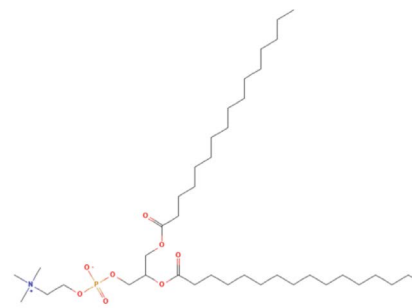
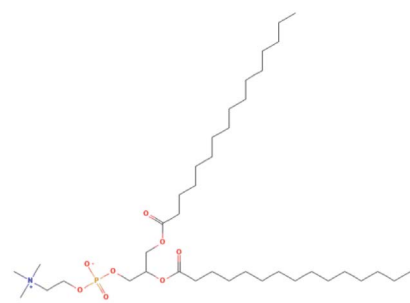
**TABLE 6.2** ***(Continued)***
**Types of Lipids Used for the Preparation of mRNA LNP**

| Sr No | Name of Lipid | Role | Structure |
|---|---|---|---|
| 15 | EDMPC [90] | Cationic lipid |  |
| 16 | DOSPER [87] | Cationic lipid |  |
| 17 | CLinDMA [91] | Cationic lipid |  |
| 18 | SM-102 [80] | Cationic lipid |  |

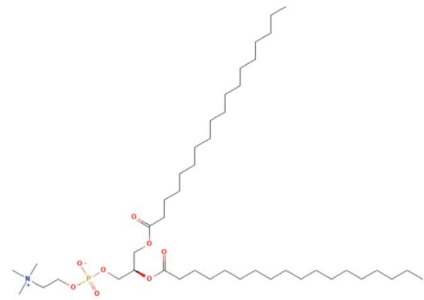
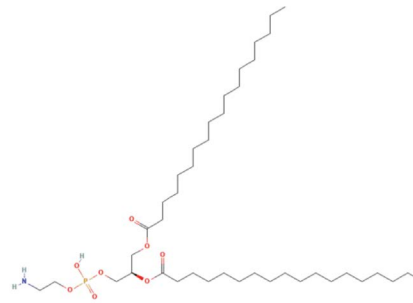
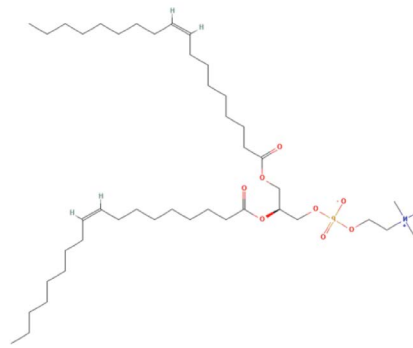
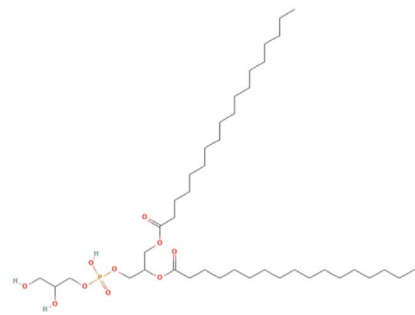
*(Continued)*

**TABLE 6.2** ***(Continued)***
**Types of Lipids Used for the Preparation of mRNA LNP**

| Sr No | Name of Lipid | Role | Structure |
|---|---|---|---|
| 19 | DMOBA (N,N-Dimethyl-3,4-dioleyloxybenzylamine) | Cationic lipid |  |
| 20 | DLinDAP [86] | Cationic lipid |  |
| 21 | 1,2-Dipalmitoyl-rac-glycero-3-phosphocholine (DPPC) [92] | Helper lipid |  |
| 22 | 1,2-Dipalmitoyl-rac-glycero-3-phosphocholine (DMPC) [93] | Helper lipid |  |

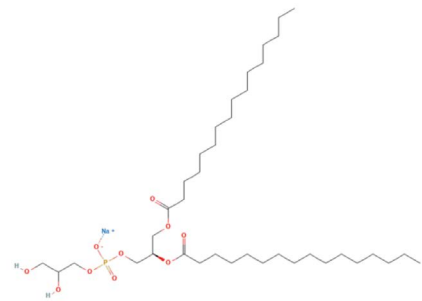
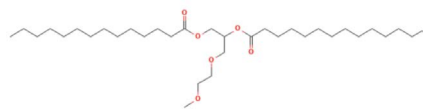
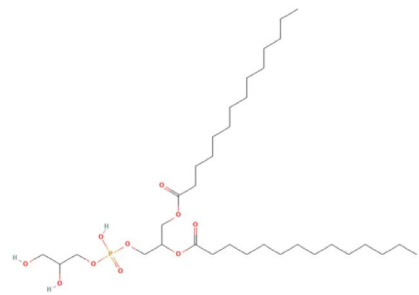
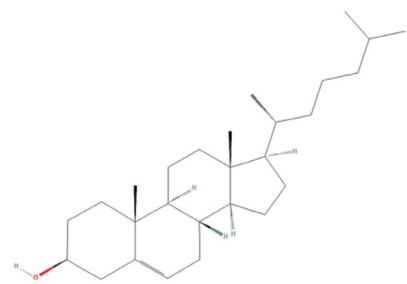
*(Continued)*

**TABLE 6.2** ***(Continued)***
**Types of Lipids Used for the Preparation of mRNA LNP**

| Sr No | Name of Lipid | Role | Structure |
|---|---|---|---|
| 23 | 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) [94] | Helper lipid |  |
| 24 | 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) [95] | Helper lipid |  |
| 25 | 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) [96] | Helper lipid |  |
| 26 | Distearoyl phosphatidylglycerol (DSPG) [97] | PEG lipid |  |

*(Continued)*

**TABLE 6.2** ***(Continued)***
**Types of Lipids Used for the Preparation of mRNA LNP**

| Sr No | Name of Lipid | Role | Structure |
|---|---|---|---|
| 27 | 1,2-Dipalmitoyl-sn-glycero-3-phospho-(1′-rac-glycerol), sodium salt (DPPG) [98] | PEG lipid |  |
| 28 | mPEG-Dimyristoyl glycerol | PEG lipid |  |
| 29 | Dimyristoylphosphatidylglycerol (DMPG) [99] | PEG lipid |  |
| 30 | Cholesterol [100] | Cholesterol |  |

Other mixing chambers and connectors with narrow relative angles, such as those between 27° and 90° or between 90° and 180°, can also be utilized. LNPs are formed instantaneously when an aqueous and organic solution mixed simultaneously and continuously together in the mixing chamber. After preparation of LNPs, they need to be diluted immediately means in less than 10 seconds to 1 minutes. An LNP

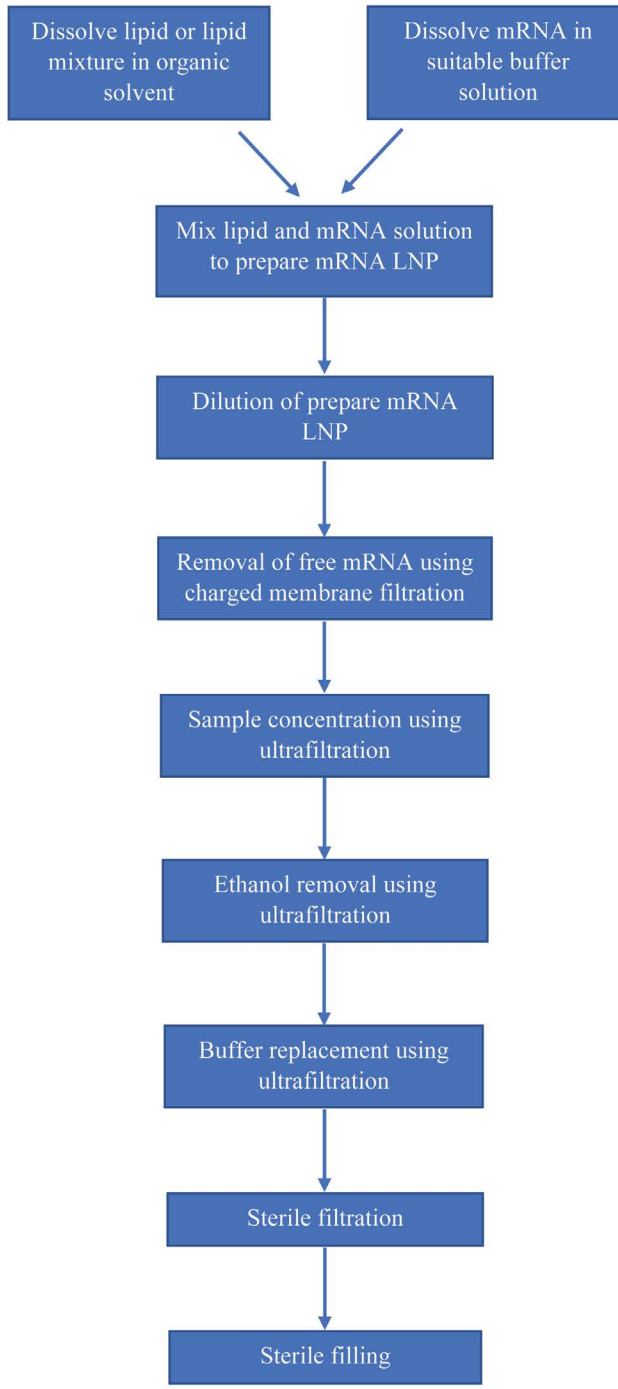



**FIGURE 6.5** Flow diagram for the preparation of LNP through direct dilution method.

prepared by this method have an entrapment efficiency of up to 90% and mean diameter of less than 150 nm [101].

iii. LNP dilution: After the LNPs formation step, the entrapment efficiency can be improved and particle size can be preserved and even decreased via instant dilution of dispersion prior to the free drug removal. For example, if entrapment efficiency of LNPs before dilution is 50–60% then it can be enhanced up to 80–90% after dilution with suitable aqueous buffer. In dilution step, the dispersion is diluted up to 10–40% of organic solvent, most preferably 20%. Optionally, an incubation can be done at the room temperature of a diluted sample [101].

iv. Removal of free drug: The free drug from the dispersion bulk can be removed using anion exchange chromatography after diluting the sample. The use of anion exchange resin offers several benefits: it provides a high dynamic capacity for nucleic acid removal, supports single-use applications, fully scalable, and can be validated and pre-sterilized [101].

v. Sample concentration: In certain cases, the dispersion solution is optionally concentrated using ultrafiltration. During concentration, a buffer solution from the dispersion is remove. An ultrafiltration can be performed utilizing cartridges packed with polysulfone hollow fibers having internal diameter of 0.5–1.0 mm and nominal molecular weight cutoff of 30,000. In certain cases, 1,000–750,000 molecular weight cutoff hollow fibers can also be employed. During ultrafiltration, a small molecules and solvents are removed from the dispersion through pores of hollow fiber while LNPs are retained with in the hollow fiber and recirculated. During the process, the filtrate collected is recognized as a permeate solution [101].

vi. Organic solvent removal: A concentrated dispersion bulk obtained in previous step is diafiltrated using aqueous buffer solution to remove an organic solvent till the concentration of organic solvent remains less than 1% in bulk. During this process, the concentration of lipid and API remain unchanged and entrapment efficiency also remains constant [101].

vii. Buffer replacement: The aqueous buffer solution is replaced with another buffer solution using same diafiltration process after removing the organic solvent. A concentration of lipid and API remains constant in this step also [101].

viii. Sterile filtration: A sterile filtration can be performed using a sterile capsule filter at a pressure of 40 psi or less. In some cases, heating jacketed vessel can also be used because ease of filtration can be achieved on slightly heating the sample [101].

ix. Sterile fill: Sterile filling will be done in class 100 area. After sterile filtration, the final product will be filled in suitable and sealed using appropriate packaging materials [101].

2. Microfluidizer-based LNP formation

A microfluidizer-based manufacturing of LNPs is widely recognized as an effective method for the industrial output. This technique relies on nanoprecipitation, achieved through the precisely controlled mixing of drug and lipid or lipid mixture solutions within microchannels. This quick microscale

mixing in microfluidizer leads to the formation of LNPs which are more uniform and reproducible in comparison to the traditional manufacturing procedures. Furthermore, microfluidic channels have a very small dimensions networks which offers the following advantages over the traditional methods like homogeneity of LNPs, better size control, and enhance mass and heat transfer [45, 102, 103]. A front view of microfluidizer system is presented in Figure 6.6. Following steps are used to prepare the LNPs using the microfluidizer:

i. Preparation of lipid and aqueous phase solutions
   a. Lipid phase: Lipid phase will be prepared by dissolving suitable lipids in organic solvent at suitable temperature. The lipids include

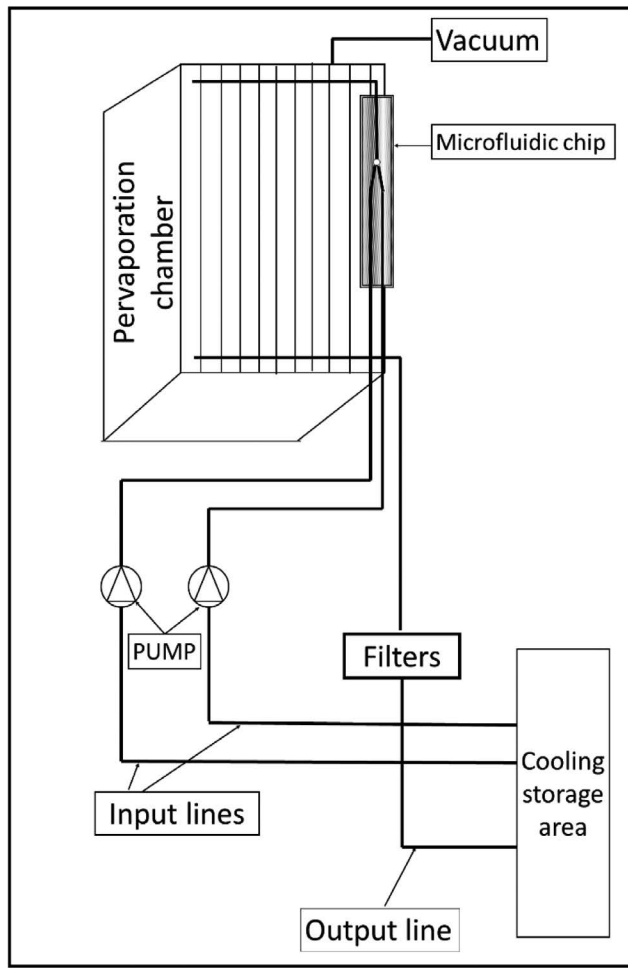



**FIGURE 6.6** Schematic diagram of formulation production system (mRNA-LNP) based on microfluidic device. This figure shows a front view of formulation production system for producing lipid nanoparticles. Storage containers for reagents and output product for downstream processes can be kept in cooling storage area.

phospholipids (helper lipids), cholesterol, PEG-modified lipids, ionizable lipids, etc. An ionizable lipid is used to encapsulate the polynucleotide, helper lipids modulate the structure of the carriers, cholesterol is used to provide strength to particle and PEG modified-lipids reduce particle aggregation. An organic solvent should be miscible with aqueous phase. Thus, an organic solvent is chosen from the group of alcohols like ethanol, methanol, propanol, isopropanol, butanol, and mixture of them [45, 102, 103].

b. Aqueous phase: Aqueous phase will be prepared by dissolving one or more polynucleotide of interest and other required excipients in suitable buffer. An aqueous solution prepared here should have an acidic pH. The term polynucleotide refers to the type of DNA and RNA [45, 102, 103].

ii. LNP preparation: A rapid microfluidic mixing of aqueous phase and organic phase will be performed in microfluidic mixer which ultimately resulted into the formation of LNP. In this process, product output can be increased by using plurality of microfluidic mixer in parallel (5–20 mixer). An aqueous phase and organic phase will be introduced to the microfluidic chamber through two separate inlet channels with the help of two different pumps. A flow rate of both phases will be kept same or different. Particle size of the prepared particles depends on a flow rate ratio of an aqueous to organic phase. Higher ratio will result in higher particle size and vice versa. Due to the miscibility of an organic solvent with water, when organic and aqueous phases are mixed, they result into homogenous monophasic solution. Lipids do not have sufficient solubility in this monophasic solution, this its start to recrystallize via nucleation and form LNPs. So, this rate of nucleation and particle growth depend on the volume ratio of aqueous to organic phase. After formation of nanoparticles, a dispersion can also be diluted if required [45, 102, 103].

iii. Solvent evaporation: An organic solvent which used to dissolved the lipids needs to remove from the final dispersion to meet the regulatory requirement. The common practice to remove the solvent from the final dispersion is tangential flow filtration (TFF), involving several cycles of concentration and dilution with aqueous buffer until the residual solvent concentration meets regulatory requirements. However, TFF can impose shear stress on the nanocarriers, negatively impacting their quality. To overcome the limitation of TFF, pervaporation technique can be used. This technique removes the organic solvent from the dispersion effectively ensuring the quality and integrity of the nanocarriers. Moreover, compared to the TFF, the pervaporation step does not require a buffer and therefore reduces process footprint and waste. Pervaporation of the prepared formulation can be performed using pervaporation device. This device contains separation membrane, so when a vacuum and/or purge gas applied to the chamber on formation, organic solvent will be removed from the

formulation and diffused through the membrane. The membrane is porous in nature, and can be prepared from the various materials like silicone (polydimethylsiloxane), cellulose, PET (polyester), or alike. A preferred purging gas is nitrogen gas. The pervaporation can be operated in a continuous fashion, provided that the surface of the membrane and the surface-to-volume ratio are large enough. Continuous processing confers several advantages, such as a decrease in equipment footprint, automatability and an ability to increase scale by scale-out of compact manufacturing lines, or extension of the processing time. A pervaporation is carried out at a temperature range of 21–35°C while maintain pressure of 0.5 mbar to 100 bar. For continuous pervaporation process, a feed rate of 10 μL/min to 500 mL/min can be utilized [45, 102, 103].

One should have to consider the following parameters before adapting this method for a preparation of mRNA LNPs which can affect the product quality drastically: pH of aqueous buffer solution, type of organic solvent used, type of lipids used, concentration of lipid used, N/P ratio, flow rate of aqueous phase and organic phase, volume ratio of aqueous to organic phase, ratio of flow rate of aqueous phase to organic phase, flow rate of purging gas, or amount of vacuum applied, etc.

3. Ethanol injection method

An mRNA-loaded LNPs preparation using this technique comprises following three steps: (i) mix an aqueous and lipid solution to obtain precursor mRNA LNP (a lipid solution contains an ionizable lipid, structural lipids, helper lipids, etc., while aqueous phase will be prepared by dissolving nucleic acid in water); (ii) then, to modify the mRNA LNPs, LNP modifier will be added; and (iii) further, both the LNPs will be processed for obtaining the nucleic acid LNP composition [104].

In an organic solvent which is miscible with water, the lipid mixture will be dissolved, like ethanol. A lipid mixture (containing ionizable lipid, first PEG, phospholipid, structure lipid) will be dissolved in absolute alcohol. An ionizable lipid should have at least one biodegradable group and a central amine moiety. A PEG lipid should be chosen from the following: a PEG-modified phosphatidic acid, a PEG-modified phosphatidylethanolamine, a PEG-modified ceramide, a PEG-modified diacylglycerol, a PEG-modified dialkylamine, a PEG-modified dialkylglycerol, etc. Incorporation of structural lipids in LNP may assist accumulation of other lipids with in the particle. From many available structural lipids, one is selected for the LNP preparation. The available structural lipids are fecosterol, cholesterol, sitosterol, campesterol, ergosterol, stigmasterol, etc. Phospholipids may assemble into one or more lipid bilayers. Some phospholipids can also facilitate fusion to a membrane. Because of this fusion, one or more contents of LNP will be able to pass through cell membrane to reach the target site. A phospholipid for the LNP manufacturing can be selected from the following: phosphatidyl choline, phosphatidyl glycerol, phosphatidyl

ethanolamine, phosphatidyl serine, 2-lysophosphatidyl choline, phosphatidic acid, and a sphingomyelin [104].

An aqueous solution was prepared by dissolving polynucleotide in suitable buffer solution. Ideally, the buffer's pH is lower than the pKa of a protonated lipid within the lipid mixture. There are various buffers which will be used for this purpose like citrate, phosphate, and acetate. The buffer concentration is influenced by the chemistry of the mRNA being encapsulated, and optimizing this concentration can be crucial for obtaining high drug loading. An osmotic potential between the particle membrane should be maintained by adding the cryoprotectant and/or non-ionic solute [104].

The modifying agent should be picked from the group comprising of a PEG lipid and a surfactant. If PEG lipid is used as a modifying agent, then it will be termed as a second PEG lipid. In some cases, first PEG lipid and second PEG lipid might be same [104].

After preparing both the phases, an LNP can be manufactured by adding an organic solution in an aqueous solution. At the time of addition of the lipid solution, an aqueous mRNA solution should be at a temperature of 25–45°C. The organic lipid solution may be added to the mRNA aqueous solution by pump or gravity at a precise rate. Multiple approaches can be used to add an organic solution in aqueous solution such as through a tube or outlet, a single spray or stream, or through a multi-outlet system. During adding the organic solution in the aqueous solution, a resulting solution must be mixed by shaking or stirring or recirculating [104].

A prepared LNP needs to be stabilized before or when stress will be applied during its production process by adding modifying agents in to it. A second PEG can be used in two ways: (1) post-insertion procedure and (2) post-addition phase. The post-insertion procedure comprised: (i) a nanoprecipitation reaction between the lipid mixture solubilized in ethanol and the mRNA in aqueous solution, (ii) exposure of the resulting particles to a solution comprising a certain weight percentage of PEG-DMG, (iii) TFF, and (iv) a final filtration step. While the post-addition procedure comprised: (i) a nanoprecipitation reaction between the lipid mixture solubilized in ethanol and the mRNA in aqueous solution, (ii) TFF, (iii) exposure of the filtered particles to a solution comprising a certain weight percentage of PEG-DMG, and (iv) a final filtration step [104].

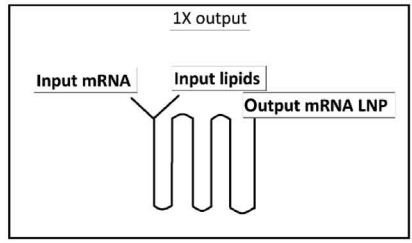
After preparation of LNPs, the dispersion will be subjected to the diafiltration process. The purpose of the diafiltration is any of the following: pH adjustment, buffer exchange, solvent removal, and concentration of LNPs. Diafiltration of the LNP dispersion will be carried out with TFF using hollow fiber cartridge or cassette. The final aqueous dispersion will be decontaminated before storage or use sterile filtration using a 0.22-μm filter. The last step of the procedure comprises a lyophilizing of the aqueous dispersion after filling in a suitable container [104].

## 6.7 RECENT ADVANCES IN LIPID NANOPARTICLE MANUFACTURING PROCESS

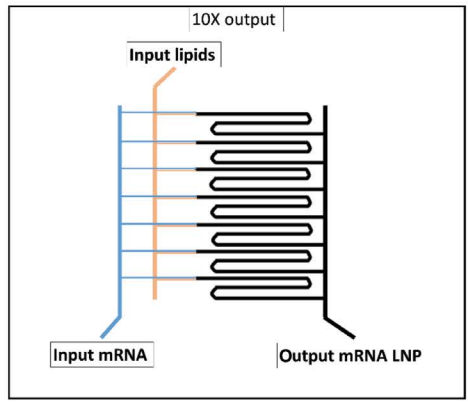
Recently, Sarah J. Shepherd et al. developed SCALAR (silicon scalable lipid nanoparticle generation) platform technology to produce mRNA-based lipid RNA (Figure 6.7). As mentioned above, to increase the output in case of microfluidic-based LNP production, multiple units of microfluidic chip need to be used. By using this technique, high manufacturing rates of >10 L/h can be achieved; however, this approach is not easily scalable to >10× chips since each chip needs to have its own macroscopic fluidic handling systems. While in case of SCALR technology, they have incorporated an array of 256 mixing units on single 100-mm microfluidic chip. They have prepared this chip out of silicon and glass substrate. They assert that their technology has the potential to reduce a time requires from LNP optimization to clinical usage. This can be achieved due to their high manufacturing rate chips with minimizing batch-to-batch variations which can be arise due to usage of different manufacturing techniques at small and large scales. Their design integrates a ladder geometry with a branching architecture, incorporating 256 staggered herringbone micromixer units onto one single silicon chip. This chip can operate at a modest pressure of 100 psi and is manufactured using conventional semiconductor processes. To distribute and collect fluids from individual mixing units, a ladder geometry is typically favored. Thus, it occupies a smaller footprint and becomes more resistant to clogging compared to a solely branched design [105].

To guarantee that each device in the array generates LNPs with consistent physical attributes, they have integrated high fluidic resistance microchannels upstream of each device. This approach decouples the design of a mixing unit from the fluidics needed for parallelization. They have developed chips with 1×, 10×, or 256× mixing units, enabling the manufacturing rate in the range of 0.1–17 L/h. This variation is achieved without altering the flow conditions or mixing geometry compared to a single 1× mixing chip. SCALAR chips are consistently reproducible in both fabrication and operation. It offers various advantages like resistant to damage or swelling by solvent, compatible with high temperature thus sterilization can be easily done and eliminate risk related to leaching from chip materials [105].

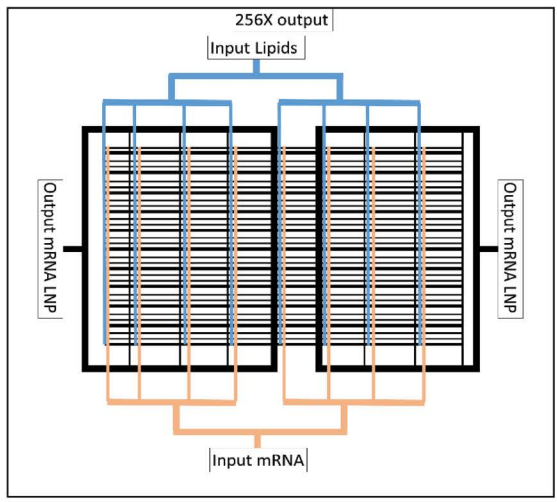
They have prepared the LNPs at a manufacturing rate of 17 L/h which is equal to the 8.5 g/h mRNA. Further, they have manufactured the LNP using all the SCALAR chips and demonstrated comparable physical properties across them. This proves the effectiveness of the silicon glass-based microfluidizer for the reproducible large-scale manufacturing. Moreover, the materials used in their chips enable them to be reset and reused. This fabrication strategy can accommodate over 20,000 equal microfluidic units, allowing the total throughput to be further enhanced by adding more mixing units to a single 100-mm microfluidic chip. This approach eliminates the need for additional fluidic handling systems. Additionally, this LNP manufacturing technique can be seamlessly combined with other processes, such as TFF or dialysis, to ease the continuous mRNA-loaded LNP production [105].




(A)




(B)




(C)

**FIGURE 6.7** A throughput-scalable SCALAR platform that incorporates 1× (A), 10× (B), or 256× (C) mixing units onto a single microfluidic chip to formulate mRNA LNPs. Scale bars are 1 mm for the 1× chip; 5 mm for the 10× chip; 10 mm for the 256× chip [105].

## 6.8 CONCLUSION

There are various techniques which can be used to prepare LNPs on a lab level. However, scale-up of these techniques from lab to commercial level is very complex process. There are various factors which can affect product quality which needs to be considered while transferring method from lab to plant level. On plant level, these factors require further optimization, due to the increase in bulk size can affect product quality adversely. So, out of many above-listed techniques only few techniques are feasible for scale-up and due to the complexity in handling of mRNA, these techniques further reduced to direct dilution method, microfluidizer-based LNP preparation, and ethanol injection method. All three techniques come under the bottom-up approach of nanoparticles preparation and require further optimization as bulk size increases which increases the product development time and delays the product timeline for market availability. There is one latest technique which can provide output of 17 L/h without much change in parameters which are optimized in laboratory level –SCALAR. However, there is no marketed product which is manufactured using this platform technology.

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# 7 Commercial Production of Liposomes

## *Advancements and Innovation in Manufacturing Processes, Quality Assurance, and Control*

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### 7.1 INTRODUCTION

Liposomes are bilayer vesicular structures primarily composed of phospholipids and cholesterol, offering a versatile platform for the encapsulation and delivery of both hydrophilic and lipophilic compounds. They have gained widespread acceptance in the pharmaceutical, cosmetic, and agricultural industries. Unlike traditional treatments, liposomal therapies are widely explored since they are biodegradable, biocompatible, and resemble lipids found in cellular membranes. Commercial liposomal products are shown in Figure 7.1.

Liposomes are spherical vesicles composed of one or more phospholipid bilayers, closely resembling biological membranes. Since their discovery in the 1960s by Bangham et al., liposomes have garnered significant attention drug delivery systems due to their ability to encapsulate both hydrophilic and hydrophobic substances [1]. The unique structural properties of liposomes allow for targeted delivery and controlled release of therapeutic agents, enhancing efficacy while minimizing side effects. They have been instrumental in the delivery of chemotherapeutic agents, antifungal drugs, and more recently, nucleic acid-based therapies [2]. Although access to these advanced treatments can be limited (mainly due to their cost), with the rising cases of breast and ovarian cancers worldwide, the global market for liposomal doxorubicin alone is accounted for USD 1.3 billion in 2023 and is expected to reach over USD 2 billion by 2032 [3].

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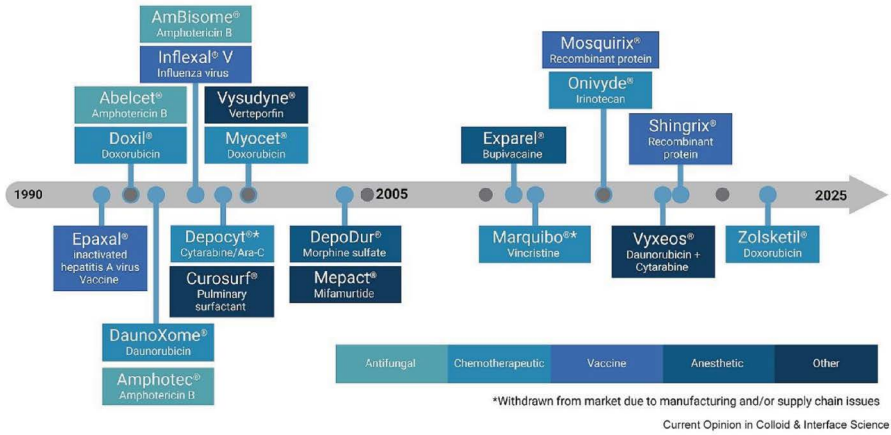


**FIGURE 7.1** Approval of liposomal therapeutics over the last three decades. (Reproduced from Liposomes: Bridging the gap from lab to pharmaceuticals by Eugster R and Luciani P, published in *Current Opinion in Colloid & Interface Science*, 2025, under the Creative Commons CC-BY license. Available at: https://doi.org/10.1016/J.COCIS.2024.101875 [7].)

### 7.1.1 Liposome Classification and Composition

Liposomes can be classified in multiple ways as given in Table 7.1.

Liposomes are spherical vesicles composed of lipid bilayers that enclose an aqueous core. They are typically formed from natural or synthetic phospholipids, which are amphiphilic molecules comprising hydrophilic head groups and hydrophobic tails. The bilayer structure is a result of the self-assembly of these lipids in aqueous environments, where the hydrophilic head groups face outward toward the water, and the hydrophobic tails are sequestered inward, away from the aqueous environment [4].

Phosphatidylcholine (PC) is one of the most commonly used phospholipids in liposome formation due to its biocompatibility and abundance in biological membranes. Other phospholipids, such as phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), may also be incorporated to modify the properties of the liposome, such as charge and stability [5]. Cholesterol is often included in liposome formulations to enhance membrane rigidity and reduce permeability, thus increasing stability and prolonging circulation time *in vivo*.

Liposome composition can be tailored to suit specific applications, such as drug delivery, imaging, or vaccine development. For instance, surface modifications with polyethylene glycol (PEG), referred to as PEGylation, can reduce opsonization by the immune system, thereby extending the liposome's circulation half-life [6]. Similarly, targeting ligands such as antibodies, peptides, or aptamers can be conjugated to the liposome surface to facilitate selective binding to specific cells or tissues, enhancing therapeutic efficacy [5].

**TABLE 7.1**
**Classification of Liposomes**

| Classification Criterion | Category | Description |
|---|---|---|
| **Size** | Small unilamellar vesicles (SUVs) | Diameter: 20–100 nm. Suitable for systemic drug delivery due to small size and stability. |
| | Large unilamellar vesicles (LUVs) | Diameter: >100 nm. Favorable for encapsulating larger drug molecules or macromolecules. |
| | Multilamellar vesicles (MLVs) | Diameter: 0.1–10 μm. Often used in topical formulations or depot systems due to slower drug release. |
| | Giant unilamellar vesicles (GUVs) | Diameter: >1 μm. Primarily used in biophysical and mechanistic studies or artificial cell models. |
| **Lamellarity** | Unilamellar | Single phospholipid bilayer enclosing the aqueous core. Useful for high drug encapsulation efficiency. |
| | Multilamellar | Multiple concentric bilayers with aqueous layers in between. Suitable for sustained-release formulations. |
| | Oligolamellar | Intermediate between unilamellar and multilamellar, consisting of a few bilayers. |
| **Charge** | Neutral | No net surface charge; reduces opsonization and immune system recognition. |
| | Cationic | Positively charged, enhancing interaction with negatively charged cell membranes, but potentially toxic. |
| | Anionic | Negatively charged, often used for targeting specific receptors or proteins. |
| | Zwitterionic | Contain both positive and negative charges; useful for balanced interactions with biological systems. |
| **Composition** | Natural lipid-based | Derived from biological sources (e.g., phosphatidylcholine, cholesterol). |
| | Synthetic lipid-based | Designed for specific properties like charge, stability, or targeting (e.g., DOTAP, DOPE). |
| | Hybrid liposomes | Incorporation of polymers (e.g., PEG) or other materials for enhanced functionality. |
| | Fusogenic liposomes | Contain lipids like DOPE to facilitate membrane fusion and intracellular delivery. |
| **Surface Modification** | PEGylated | Coated with polyethylene glycol (PEG) to reduce clearance by the mononuclear phagocyte system. |
| | Ligand-modified | Functionalized with targeting ligands like antibodies, peptides, sugars, or aptamers for active targeting. |
| | Stimuli-responsive | Modified to respond to external or internal stimuli (e.g., pH, temperature, enzymes) for controlled release. |
| | Magnetic liposomes | Embedded with magnetic materials for targeted delivery and imaging using magnetic fields. |

The ability to encapsulate hydrophilic drugs within the aqueous core and hydrophobic drugs within the lipid bilayer further highlights the versatility of liposomes as drug delivery systems. Their composition and functionality can be precisely engineered to overcome biological barriers, optimize drug release profiles, and minimize off-target effects, underscoring their importance in modern biopharmaceuticals [7].

## 7.2 METHODS OF LIPOSOME PREPARATION

The initial methods for liposome preparation were labor-intensive and not suitable for large-scale production. Table 7.2 gives overview of different lab-scale methods used for liposome preparation.

Techniques such as thin-film hydration and sonication were commonly used in laboratory settings [8]. With the growing demand for liposomal products, there was a necessity to develop scalable and reproducible manufacturing processes. Innovations such as high-pressure homogenization and extrusion methods emerged to address these challenges [9]. The transition from laboratory-scale to commercial-scale production is a significant milestone, requiring innovations in manufacturing technologies and stringent quality assurance (QA) measures. The further sections explore different liposome production technologies, critical quality attributes (CQAs), and emerging trends in the field.

### 7.2.1 Traditional Methods

#### 7.2.1.1 Thin-Film Hydration

This method involves dissolving lipids in an organic solvent, forming a thin film upon solvent evaporation, followed by hydration with an aqueous phase. While simple, it suffers from scalability issues [9]. The thin-film hydration method is a well-established technique for the preparation of liposomes, widely used in both laboratory and commercial settings. This method involves the deposition of a thin lipid film on the inner wall of a round-bottom flask, which is subsequently hydrated with an aqueous phase. The lipid film is typically formed by dissolving lipids in an organic solvent, such as chloroform or methanol, which is then evaporated under reduced pressure using a rotary evaporator. Once the solvent is completely removed, the resulting thin lipid layer is rehydrated with an aqueous solution, often containing the active pharmaceutical ingredient (API), under controlled conditions. This hydration process leads to the self-assembly of lipids into bilayer vesicles, forming liposomes [4].

Scaling up the thin-film hydration method for commercial applications presents unique challenges, particularly in achieving consistent size distribution, encapsulation efficiency, and batch-to-batch reproducibility. To address these issues, modifications to the standard procedure and the use of advanced equipment have been introduced. For instance, the hydration step can be enhanced through mechanical agitation, such as vortexing or sonication, to improve lipid dispersion and vesicle uniformity [10]. Despite its advantages, including simplicity and the ability to encapsulate both hydrophilic and hydrophobic drugs, the thin-film hydration method has limitations at commercial scales. These include difficulties in achieving high

**TABLE 7.2**
**Lab-Scale Methods for Preparation of Liposomes**

| Method | Process | Advantages | Limitations | Applications |
|---|---|---|---|---|
| **Thin-Film Hydration** | Lipid film formation followed by hydration with an aqueous solution. | Simple, cost-effective, widely used for initial formulation studies. | Low scalability, requires size reduction via extrusion or sonication for uniformity. | General research, proof-of-concept studies. |
| **Reverse-Phase Evaporation** | Emulsification of lipid in organic and aqueous phases, followed by solvent evaporation. | High encapsulation efficiency for hydrophilic drugs, suitable for complex formulations. | Time-consuming, difficult to scale up due to solvent removal requirements. | Hydrophilic drug delivery studies. |
| **Ethanol Injection** | Lipid solution in ethanol injected into an aqueous buffer to form liposomes. | Rapid, straightforward, and does not require specialized equipment. | Residual ethanol removal is necessary, limited control over size and polydispersity. | Small molecule drug delivery formulations. |
| **Microfluidics** | Controlled mixing of lipid and aqueous phases in a microfluidic device. | High precision, reproducibility, and control over liposome size; minimal solvent use. | Low throughput, requires specialized devices. | Gene therapy, nanoparticle research. |
| **Sonication** | Application of ultrasound waves to reduce liposome size. | Effective for reducing size of multilamellar vesicles (MLVs) to small unilamellar vesicles (SUVs). | Potential degradation of sensitive materials, limited scalability. | Small-scale production of SUVs for drug delivery. |
| **Extrusion** | Lipid suspension is passed through membranes with defined pore sizes. | Produces liposomes with uniform size and high reproducibility. | Time-intensive, not suitable for large-scale applications without automation. | Size-controlled liposome production. |
| **Detergent Removal** | Detergent-solubilized lipids are dialyzed to form liposomes. | Allows incorporation of membrane proteins and controlled reconstitution of lipids. | Time-intensive, requires extensive dialysis to remove detergent. | Membrane protein studies, specialized liposomes. |

encapsulation efficiency for certain drugs and the labor-intensive nature of the process. To mitigate these issues, Wang et al. proposed a scalable modification to the traditional methodology which involves forming a proliposomal solid lipid film that can be directly hydrated within a vial [11]. By subjecting the vial to vigorous oscillations, they achieved the production of uniform liposomes without the necessity for further size reduction steps, demonstrating feasibility at an industrial scale. Despite

its advantages, this approach may face limitations concerning total lipid concentration as it could pose difficulties for drug formulations that demand higher lipid concentrations.

#### 7.2.1.2 Reverse-Phase Evaporation

The reverse-phase evaporation (RPE) method is a well-established technique for preparing liposomes with large internal aqueous space particularly for encapsulating hydrophilic drugs and macromolecules. Originally developed by Szoka and Papahadjopoulos in 1978, this method involves the creation of a water-in-oil emulsion followed by the removal of the organic solvent to yield stable liposomes with high encapsulation efficiency [12]. At a commercial scale, the RPE method has been refined to ensure reproducibility, scalability, and compliance with stringent QA standards.

The process begins with the dissolution of phospholipids and other lipophilic components in an organic solvent, such as diethyl ether, isopropyl ether, or chloroform. The aqueous phase containing the drug or solute of interest is subsequently introduced, and the mixture is subjected to vigorous agitation, often through sonication or homogenization, to create a stable water-in-oil emulsion. The next critical step is the evaporation of the organic solvent under reduced pressure, typically using a rotary evaporator or similar systems, leading to the formation of a gel-like intermediate. This intermediate is then subjected to hydration and vortexing, resulting in the generation of unilamellar or oligolamellar liposomes depending on the specific conditions employed. This method allows higher encapsulation efficiencies for hydrophilic drugs but is limited by residual solvent issues [13].

Scaling up the RPE method for commercial production introduces several challenges and opportunities for innovation. One primary challenge is the management of solvent removal to ensure complete elimination of the organic phase, which is essential for regulatory compliance and product safety. Advanced technologies, such as continuous-flow reactors and automated solvent recovery systems, have been integrated into commercial setups to address these issues. Moreover, the choice of solvents and their compatibility with industrial-scale operations are crucial factors that influence both the efficiency and environmental impact of the process.

Another challenge is due to large organic solvent consumption and lengthy processes [14]. Novel techniques like the supercritical reverse phase evaporation method using supercritical $CO_2$ have demonstrated the formation of large unilamellar liposomes with high trapping efficiency without toxic organic solvents [15].

#### 7.2.1.3 Detergent Depletion

The detergent depletion method is a well-established technique for preparing liposomes, particularly unilamellar vesicles, through the controlled removal of detergent from a lipid-detergent mixture. This method leverages the ability of detergents to solubilize lipids into mixed micelles, which upon detergent removal, reorganize into bilayer structures forming liposomes. The process has been extensively utilized at

the laboratory scale due to its simplicity and effectiveness in producing liposomes with defined sizes and homogeneity [16].

At the laboratory scale, the method begins with dissolving phospholipids in a detergent solution at concentrations above the critical micelle concentration (CMC), resulting in the formation of mixed micelles. Common detergents used include octyl glucoside, Triton X-100, and sodium cholate, chosen for their suitable CMCs and ease of removal. The subsequent step involves the gradual removal of the detergent, allowing lipids to self-assemble into liposomal structures. Techniques such as dialysis, gel filtration chromatography, and adsorption are employed for detergent removal.

Dialysis is widely used due to its simplicity, where the lipid-detergent mixture is placed in a dialysis bag and immersed in a large volume of buffer, facilitating the diffusion of detergent molecules out of the mixture [17]. However, transitioning the detergent depletion method to commercial-scale production introduces several challenges. The scalability of detergent removal processes is a primary concern. Dialysis and gel filtration become less practical due to increased processing times and equipment limitations at larger volumes. Additionally, ensuring consistent liposome size distribution and complete removal of detergents to meet regulatory standards is more complex at large scale.

#### 7.2.1.4 Ethanol Injection

The ethanol injection method has emerged as a promising technique for the commercial-scale preparation of liposomes. This method involves the rapid injection of a lipid solution dissolved in ethanol into an aqueous medium, leading to the spontaneous formation of liposomes due to the dilution of ethanol and the self-assembly of lipid molecules into bilayers [18].

Scaling up the ethanol injection method from laboratory to commercial scale is not free from challenges, primarily related to controlling liposome size distribution and encapsulation efficiency. Parameters such as the rate of lipid solution injection, the concentration of lipids, and the mixing dynamics become critical [19]. To address these issues, process optimization is essential to maintain the quality and consistency of liposomal products [20, 21].

Studies have demonstrated successful scale-up of this method using micro-engineered membranes, producing consistent liposome sizes across different production scales [22, 23]. Economic analysis suggests that a plant with a daily production capacity of 288 L of liposomal suspension is economically feasible [20]. Furthermore, liposomes produced by this method have shown potential for pulmonary drug delivery, with aerodynamic properties suitable for deep lung deposition [23]. Advancements in equipment and process engineering have facilitated the commercialization of the ethanol injection method [24]. The use of high-shear mixers and in-line injection systems allows for better control over mixing and liposome formation [25]. Continuous-flow systems have been developed to replace batch processes, enhancing scalability and reducing variability between batches [26]. These systems enable precise control over the mixing of ethanol and aqueous phases, resulting in liposomes with uniform size and improved encapsulation efficiencies.

Another critical aspect of scaling up is the removal of residual ethanol from the liposome suspension (due to azeotropic ethanol/water mixture formation) as residual solvents can be detrimental to product stability and safety. Techniques such as freeze-drying were employed to effectively reduce ethanol content to acceptable levels [27]. Integrating these purification steps into the production process ensures compliance with regulatory standards for pharmaceutical products.

### 7.2.2 Modern Techniques

#### 7.2.2.1 Microfluidics

Microfluidics has emerged as a cutting-edge approach for the production of liposomes, offering precise control over liposome size, composition, and encapsulation efficiency [28]. This technology operates on the manipulation of fluids within microchannels, typically at the microliter scale, allowing for the generation of consistent and reproducible lipid nanoparticles [29]. Unlike conventional bulk methods, microfluidics provides a high degree of control over the mixing process, enabling the fine-tuning of liposome properties to meet specific therapeutic and industrial requirements [30].

In microfluidic systems, liposome production involves the controlled mixing of lipid-containing organic solvents and aqueous phases within microchannels. Lipids dissolved in organic solvents, such as ethanol or isopropanol, are introduced through one inlet of the microfluidic chip, while the aqueous phase containing hydrophilic drugs or other solutes is introduced through another inlet. These streams converge in a mixing region where rapid and controlled diffusion occurs due to the laminar flow regime characteristic of microfluidic systems. This controlled mixing facilitates the self-assembly of lipids into bilayers, forming liposomes with uniform size and morphology [31]. Critical parameters influencing liposome production include the flow rate ratio (FRR) of the organic to aqueous phase, which impacts liposome size and polydispersity; the total flow rate (TFR), affecting mixing dynamics and throughput; the lipid concentration in the organic phase, which determines encapsulation efficiency; and the microchannel design, which enhances mixing efficiency and controls liposome characteristics [32]. Different types of microfluidic channel designs that are used for liposome manufacturing are schematically shown in Figure 7.2.

Recent advancements in microfluidic designs have allowed the development of parallelized and continuous-flow systems, making it possible to scale up production for industrial use. Furthermore, microfluidic systems significantly reduce production time due to rapid mixing and self-assembly within microchannels. These systems also require minimal organic solvents, reducing environmental impact and simplifying downstream processing. The controlled environment in microfluidic devices allows for high encapsulation efficiencies, particularly beneficial for hydrophilic drugs [33].

Liposomes produced using microfluidics have found applications in diverse fields. In drug delivery, they facilitate the encapsulation of small molecules, peptides, and nucleic acids for targeted therapies. They are also employed in gene therapy, especially for delivering RNA and DNA molecules in mRNA-based vaccines and

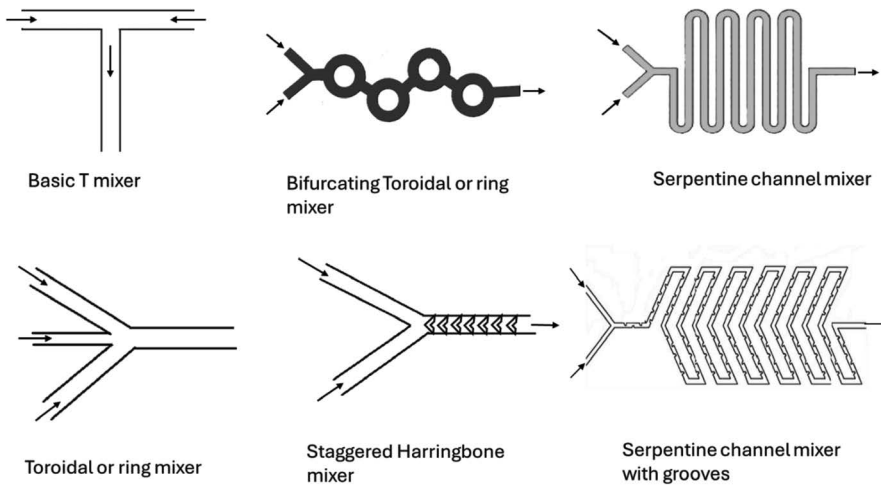


**FIGURE 7.2** Schematic representation of different designs of microfluidic channels.

CRISPR-based therapeutics [34]. Despite its advantages, microfluidic liposome production faces challenges, including device fouling due to lipid accumulation, high initial costs associated with specialized fabrication, and throughput limitations, though scalable designs are emerging [35]. Future advancements in microfluidic technology, such as integrating in-line monitoring and feedback systems, could further enhance its efficiency and versatility [36]. Moreover, combining microfluidics with artificial intelligence and machine learning tools may allow for the real-time optimization of production parameters, ensuring high-quality liposomes tailored to specific applications [37].

Microfluidics thus represents a transformative technology for liposome production, providing unparalleled precision and flexibility. As developments in device design and scalability continue, microfluidics is poised to become a cornerstone method for manufacturing liposomes, both in academic research and industrial applications.

#### 7.2.2.2 Supercritical Fluid Technology

Supercritical fluid technology (SCFT) has gained significant attention as an innovative and environmentally friendly approach for the large-scale production of liposomes. Supercritical fluids, particularly carbon dioxide ($CO_2$), possess unique properties that allow them to function as solvents with tunable density, viscosity, and diffusivity. These attributes make SCFT a highly versatile and efficient method for liposome manufacturing, offering advantages in terms of scalability, environmental sustainability, and process precision [38].

Supercritical $CO_2$ is the most commonly used fluid in SCFT due to its moderate critical temperature (31.1°C) and pressure (7.38 MPa), nontoxic nature, and low cost [39]. In the production of liposomes, SCFT typically involves dissolving lipids in a supercritical $CO_2$ phase, sometimes in combination with co-solvents like ethanol, to

enhance lipid solubility. The lipid-loaded supercritical phase is then mixed with an aqueous phase under controlled conditions, promoting the self-assembly of lipids into bilayers and forming liposomes. The process is highly tunable, with variables such as pressure, temperature, and the flow rates of the $CO_2$ and aqueous phases dictating the size, lamellarity, and encapsulation efficiency of the resulting liposomes [40, 41].

Several techniques utilizing supercritical fluids have been developed for liposome production. Supercritical anti-solvent (SAS) method involves spraying the lipid solution into a supercritical $CO_2$ environment, where rapid solvent extraction drives lipid precipitation and liposome formation [42]. Alternatively, the rapid expansion of supercritical solutions (RESS) method employs the rapid depressurization of a supercritical lipid solution through a nozzle, causing liposome self-assembly upon contact with an aqueous phase [43]. The particles from gas-saturated solutions (**PGSS®**) method use supercritical $CO_2$ to saturate lipid solutions, enabling liposome formation upon depressurization [44].

The method is inherently scalable, as the continuous-flow design of supercritical reactors allows for the seamless transition from laboratory-scale to industrial-scale production. Furthermore, SCFT eliminates the need for toxic organic solvents traditionally used in liposome production, making the process environmentally benign and reducing regulatory challenges. The precise control over operating conditions enables the production of liposomes with narrow size distributions and high encapsulation efficiencies, meeting stringent quality standards for pharmaceutical applications [10]. Additionally, SCFT is highly energy-efficient due to the mild temperatures required for supercritical $CO_2$, preserving the integrity of temperature-sensitive bioactive compounds such as proteins, peptides, and nucleic acids.

Applications of SCFT-produced liposomes span a wide range of fields. In pharmaceuticals, these liposomes are employed for encapsulating hydrophilic and hydrophobic drugs, ensuring controlled release and improved bioavailability. SCFT is also used in the food and cosmetics industries for delivering active compounds, flavors, and nutraceuticals in stable lipid-based formulations. Moreover, SCFT has demonstrated its utility in producing functionalized liposomes for gene therapy, vaccine delivery, and imaging applications [10].

Despite its potential, SCFT faces challenges, particularly in optimizing lipid solubility in supercritical $CO_2$ and controlling vesicle properties during the depressurization phase. Moreover, the initial investment in specialized equipment and the need for precise parameter control may increase the upfront costs of SCFT systems. However, ongoing advancements in reactor design, process modeling, and lipid-solvent interactions continue to address these limitations, making SCFT increasingly viable for industrial applications [39].

#### 7.2.2.3 High-Pressure Homogenization

This method forces a lipid solution through a narrow gap at high pressures, producing uniform liposomes suitable for large-scale production. High-pressure homogenizers are widely employed for the preparation of liposomes and lipid dispersions due to their ability to disrupt vesicle structures. Typically, the starting material is a suspension of multilamellar liposomes, which are reduced in

size within the device by subjecting the suspension to high-energy input. During processing, the sample is injected at a consistent, elevated pressure into a specially designed chamber of the homogenizer. Within this chamber, structural rearrangement of the liposomes occurs as a result of turbulence, cavitation, and shear forces. The design of the homogenization chamber varies among models offered by leading manufacturers. Some homogenizers utilize a dynamic valve, while others employ fixed geometries or a combination of dynamic and static homogenizing valves.

These instruments typically operate at maximum pressures of approximately 30,000 psi, although certain models can reach pressures as high as 60,000 psi, enabling the processing of samples with phospholipid concentrations exceeding 150 mg/mL [45]. Additionally, many small-scale homogenizers are designed with corresponding scaled-up models, facilitating the direct transfer of experimental results from laboratory settings to industrial production [46].

The characteristics of liposomes produced via high-pressure homogenization are influenced by two main sets of parameters: device-related factors and sample-specific attributes. For a given homogenizer with a specific chamber design, the applied pressure and the number of processing cycles are critical in determining the size distribution of the resulting liposomes. Sample-related factors include phospholipid composition and concentration, the initial size distribution and lamellarity of liposomes, as well as the temperature and the composition and ionic strength of the bulk medium [47]. Table 7.3 provides the details of methods used in large-scale liposome production.

### 7.2.3 Size Reduction

Size reduction methods are pivotal in refining the properties of liposomes for effective drug delivery applications. The membrane extrusion technique involves the passage of polydisperse vesicles through polycarbonate membranes with defined pore sizes, maintained above the lipid's phase transition temperature (Tm). Extrusion of liposomes through polycarbonate filters with defined pore sizes requires moderate pressures, typically ranging from 200 to 800 lb/in$^2$. This method produces monodisperse small vesicles and is often combined with freeze-thaw cycles to reduce vesicle lamellarity. While extrusion allows precise control over particle size, it faces limitations such as scalability issues and the potential for membrane pore clogging.

During extrusion, the concentric layers of multilamellar vesicles (MLVs) are compressed as they pass through the filter pores under pressure, leading to a process of membrane rupture and resealing [48]. This mechanism can result in leakage of encapsulated solutes. To minimize such losses, extrusion should be performed in a medium containing the desired final solute concentration, with the removal of external (unencapsulated) solutes once the sizing process is complete.

Membrane rupture and resealing during extrusion may also produce nonspherical vesicles, such as oval- or sausage-shaped morphologies, depending largely on osmotic forces [49]. As vesicles are forced through the pores, they elongate and lose internal volume due to transient membrane rupture, accommodating the increased surface area-to-volume ratio associated with the nonspherical shapes. Upon exiting

**TABLE 7.3**
**Large-Scale Methods for Preparation of Liposomes**

| Method | Process | Advantages | Limitations |
|---|---|---|---|
| **Large-Scale Thin-Film Hydration** | Lipid film formation and hydration using automated systems. | Established process, scalable with proper equipment modifications. | Requires additional steps (e.g., extrusion) for uniformity; lower throughput compared to other methods. |
| **High-Pressure Homogenization** | Lipid and aqueous phases forced through narrow channels at high pressure. | Produces uniformly sized liposomes with scalable throughput; suitable for continuous processing. | High-energy input can degrade sensitive components; expensive equipment. |
| **Microfluidics at Scale** | Continuous mixing of lipid and aqueous solutions in high-throughput devices. | Precise control over particle size and reproducibility; suitable for GMP-compliant production. | High initial investment; specialized knowledge and equipment required. |
| **Supercritical Fluid Technology** | Lipids dissolved in supercritical $CO_2$ mixed with aqueous phase. | Eco-friendly, solvent-free, high encapsulation efficiency. | High cost and complexity of supercritical $CO_2$ systems. |
| **Ethanol Injection** | Lipids dissolved in ethanol injected into aqueous solution with controlled mixing. | Simple, scalable, suitable for continuous production. | Requires removal of residual ethanol to meet regulatory standards. |
| **Spray Drying** | Lipid and drug solutions sprayed and dried into liposome powders. | Produces dry liposome formulations with extended shelf life. | Not suitable for all drugs; potential stability issues for sensitive molecules. |
| **Freeze-Drying (Lyophilization)** | Liposomes are frozen and dried under vacuum to remove water. | Extends shelf life; good for sensitive drugs that require cold storage. | Expensive; requires cryoprotectants to maintain liposome integrity. |

the pore, the membrane attempts to return to a spherical configuration, which is thermodynamically favorable. However, the presence of impermeable or semipermeable solutes, such as common buffers and salts, generates osmotic resistance that prevents the vesicles from fully regaining their spherical shape, resulting in the formation of elongated structures. In contrast, vesicles formed in pure water typically adopt a spherical morphology due to the absence of such osmotic constraints [49].

High-shear homogenization presents a scalable alternative by forcing liposome suspensions through a narrow orifice at high pressure, generating collisions that fragment the vesicles. While effective for large-scale production, this technique may result in a broader size distribution and risks contamination from metallic or oil residues. Homogenization pressure and number of passes are the main factors contributing to the particle size reduction. Increasing pressure and the number of processing cycles during extrusion reduce the particle size and polydispersity index (PDI), thereby enhancing the uniformity of the liposomal population. However, these adjustments

also lead to a decline in encapsulation efficiency, likely due to solute leakage caused by repeated membrane rupture and resealing events during processing [47].

Sonication employs ultrasonic energy to convert MLVs into small unilamellar vesicles (SUVs). Probe sonication delivers direct energy to the dispersion but carries higher contamination risks, whereas bath sonication is more controlled and minimizes such risks. Despite its simplicity, sonication often leads to lower encapsulation efficiency and potential degradation of liposomal components. Recent advancements, such as the work by Hoseini et al., have utilized machine learning to optimize sonication parameters, achieving desirable particle sizes with improved encapsulation efficiency [50].

### 7.2.4 Downstream Processing and Preservation

#### 7.2.4.1 Purification

Purification remains a major challenge in the development of liposomal products. Regardless of the production technique employed, it is crucial to remove nonentrapped contaminants such as small-molecule drugs or proteins from the final liposomal formulation. Commonly, separation is achieved through filtration techniques, particularly tangential flow filtration (TFF), or ultracentrifugation. However, ultracentrifugation is often impractical for large-scale applications [51]. Other purification methods include dialysis, gel-permeation chromatography, ion-exchange chromatography, and size-exclusion chromatography. These processes, though effective, are time-consuming, resource-intensive, and can reduce product yield due to dilution caused by column equilibration, particularly with size-exclusion chromatography [51]. To address these limitations, Dimov et al. proposed a continuous on-chip purification process. This approach integrates microfluidic production with TFF, enabling simultaneous liposome manufacture, drug loading, and the removal of contaminants (e.g., unentrapped drugs, proteins, and solvent residues). This continuous mode of operation offers a more efficient pathway for manufacturing, purifying, and concentrating liposomal drug products [51].

#### 7.2.4.2 Surface Modification

Surface modification of liposomes represents a crucial advancement in the field of drug delivery, enabling the development of sophisticated formulations for targeted and controlled therapeutic delivery. This process involves altering the surface characteristics of liposomes by introducing functional groups, polymers, or ligands to meet specific therapeutic requirements. The modifications are designed to improve circulation time, achieve active targeting, or evade immune system clearance, thereby enhancing the overall performance of liposomal formulations.

One of the most common approaches in surface modification is polyethylene glycol (PEG)ylation, wherein polyethylene glycol chains are covalently attached to the liposome surface. PEGylation forms a hydrophilic steric barrier that reduces protein adsorption and subsequent recognition by the mononuclear phagocyte system, thus prolonging the circulation half-life of liposomes. PEGylated liposomes, such as Doxil® (doxorubicin-loaded liposomes), have demonstrated remarkable success in clinical applications and have set benchmarks for other formulations [52].

Another strategy involves the functionalization of liposomes with targeting ligands, such as antibodies, peptides, or small molecules, to facilitate active targeting to specific tissues or cells. For example, the conjugation of folic acid to liposomal surfaces has been widely explored to target cancer cells overexpressing folate receptors. Similarly, antibodies like trastuzumab have been conjugated to liposomes for targeting HER2-positive breast cancer, showcasing the potential of ligand-modified liposomes in precision medicine [53]. However, scaling up such modifications to meet commercial production demands presents challenges, including maintaining ligand activity and achieving uniform functionalization.

Surface modification using stimuli-responsive materials is another innovative approach, wherein the liposomal surface is engineered to respond to specific stimuli such as pH, temperature, or enzymes. These modifications enable controlled drug release at the target site while minimizing off-target effects. For instance, pH-sensitive liposomes designed to release their payload in the acidic microenvironment of tumors have shown promising preclinical and clinical outcomes [54].

From a commercial manufacturing perspective, the scalability of surface-modified liposomes depends on the robustness and reproducibility of the modification processes. Challenges such as batch-to-batch variability, process optimization, and regulatory compliance must be addressed to ensure product quality and efficacy. Advanced manufacturing technologies, including microfluidic systems and automated conjugation platforms, have been developed to streamline these processes, enabling precise and reproducible surface modifications at an industrial scale [55]. Further details on the PEGylation and other stealthing technologies are covered in detail in Chapter 5.

#### 7.2.4.3 Sterilization

Conventional sterilization methods, such as heat treatment, gamma irradiation, and ethylene oxide exposure, can significantly compromise the physicochemical and biopharmaceutical properties of liposomes. These processes often result in drug leakage, phospholipid degradation, and vesicle aggregation. Even widely accepted methods like sterile filtration and aseptic processing have limitations, particularly in removing smaller contaminants, including certain bacteria and viruses [56].

Supercritical carbon dioxide (SC $CO_2$) has emerged as a promising alternative for sterilizing sensitive products like liposomes. SC $CO_2$ is nonflammable, nontoxic, environmentally sustainable, and capable of inactivating microbial contaminants with relatively simple equipment. Delma et al. demonstrated the feasibility of SC $CO_2$ in a one-step process for both producing and sterilizing liposomes, using a quality-by-design (QbD) approach to optimize process parameters [57]. However, SC $CO_2$ may not be suitable for heat-sensitive drugs and excipients and can lead to higher polydispersity compared to microfluidic methods. As a result, sterile filtration remains a reliable, cost-effective option for liposomal products with diameters smaller than 200 nm.

#### 7.2.4.4 Preservation

Preservation strategies for liposomes involve considerations of stability, shelf life, and storage conditions. The lipid composition plays a crucial role in determining liposomal stability, particularly in maintaining particle size. Other factors, such as storage temperature and buffer pH or composition, also affect stability, albeit to a lesser extent.

Lyophilization with the excipients that protect liposomes from oxidation or coalescence has proven to be a particularly attractive approach for enhancing shelf life and simplifying storage and supply chains [58]. Lyophilization, however, presents its own challenges. During the process, liposomes undergo dehydration, resulting in significant membrane interactions that can lead to destabilization. Dehydration causes the lipid bilayers to compress laterally, potentially leading to membrane fusion, aggregation, or phase separation of lipid components, compromising liposome integrity. Cryoprotectants such as glycerol, ethylene glycol, mannitol, and dimethyl sulfoxide mitigate these effects by lowering the freezing point and reducing ice crystal formation, thereby maintaining bilayer fluidity and preventing damage [58]. Lyoprotectants like sucrose and trehalose stabilize liposomal membranes during dehydration by forming a glass-like matrix around the liposomes [59]. This matrix prevents close packing, reducing the risk of fusion and aggregation. Trehalose, in particular, interacts with phospholipid head groups, substituting for water molecules to preserve bilayer structure and maintain membrane integrity during storage. Recently, gamma-cyclodextrin has been proposed as a novel protectant for complex formulations delivering DNA plasmids and conjugated antibodies, offering further advancements in liposome preservation [60].

### 7.2.5 Process Optimization and Scale-Up Challenges

Scaling up liposome production from laboratory to industrial levels involves intricate process optimization to ensure product quality, consistency, and regulatory compliance. Central to this endeavor is the identification and control of critical process parameters (CPPs), critical formulation parameters, and CQAs, all of which play pivotal roles in determining the safety and efficacy of liposomal drug products. Furthermore, the integration of process analytical technology (PAT), as emphasized by the FDA's guidance, has emerged as a transformative approach to real-time monitoring and control of manufacturing processes [61]. PAT not only supports the monitoring of CQAs and CPPs but also facilitates regulatory compliance through adherence to QbD principles. Real-time data acquisition allows manufacturers to identify deviations early and make adjustments proactively, minimizing waste and improving batch-to-batch consistency. These capabilities are particularly valuable in complex manufacturing setups, such as microfluidic systems or high-pressure homogenization, where small variations can have significant impacts on final product quality. By integrating PAT with automation and advanced data analytics, manufacturers can achieve a deeper understanding of process performance, supporting robust scale-up and efficient troubleshooting [62].

To address regulatory and quality challenges, manufacturers must adhere to guidelines outlined by the International Council for Harmonisation (ICH Q8) and regulatory agencies like the FDA. These guidelines emphasize a QbD approach, where the relationship between CPPs, formulation parameters, and CQAs is established through systematic studies. Real-time monitoring and data analytics can provide deeper insights into process performance, enabling proactive adjustments to maintain product quality [63].

One of the foremost challenges in scale-up is maintaining uniformity in liposome size and encapsulation efficiency, which are crucial CQAs [64]. Liposome size impacts biodistribution, cellular uptake, and clearance, while encapsulation

efficiency determines drug loading and therapeutic efficacy. In microfluidics-based production, flow rates and mixing ratios are critical CPPs, directly influencing liposome size distribution. During high-pressure homogenization, pressure settings and cycle numbers must be carefully optimized to achieve consistent size reduction without compromising the integrity of encapsulated drugs, particularly heat- or shear-sensitive molecules. Similarly, ethanol injection methods require precise control of the lipid-to-ethanol ratio and injection speed to produce liposomes with reproducible sizes and high encapsulation efficiencies. Here, the use of real-time PAT, such as dynamic light scattering (DLS) for size analysis, has become indispensable for monitoring these parameters in large-scale manufacturing [65].

Formulation challenges also arise during scale-up, particularly in achieving reproducibility across batches. Critical formulation parameters, such as lipid composition, drug-to-lipid ratio, and pH of the aqueous phase, must be tightly controlled. Variations in lipid quality or source can lead to differences in bilayer properties and drug encapsulation. These issues can be mitigated by rigorous raw material characterization and supplier qualification. PAT tools like Raman spectroscopy can be used for real-time characterization of lipid compositions and verification of drug loading [66]. Additionally, the use of design of experiments (DoE) during the formulation stage can help identify optimal parameter ranges, minimizing variability during commercial production [63, 64].

A significant hurdle in scale-up is solvent removal, particularly for ethanol injection methods, where residual ethanol levels must meet stringent regulatory limits. Advanced solvent recovery systems and continuous purification techniques, such as diafiltration, can address this issue. Similarly, stability is a critical CQA, with liposomal formulations prone to aggregation, drug leakage, or oxidation during storage. To address this, different approaches like freezing [67], freeze-drying [67], proliposomes [68], and stabilizing agents like cholesterol [69] are used.

Temperature, another critical CPP, is particularly relevant for processes involving heat-sensitive drugs or mRNA-based liposomes, such as those used in COVID-19 vaccines. Precise temperature control during lipid hydration, drug encapsulation, and storage is essential to maintain product stability. The integration of automation and control systems, such as programmable logic controllers (PLCs), can facilitate consistent temperature regulation during scale-up. Furthermore, process validation through pilot-scale studies ensures that optimized conditions are reproducible and meet regulatory standards [70]. Lyophilization of these formulations could be an effective strategy for stability [71, 72].

### 7.2.6 Production and Quality Control

The production of liposomes, a versatile drug delivery system, requires stringent QA and quality control (QC) measures to meet regulatory standards and ensure product safety, efficacy, and consistency. CQAs are defined as the physical, chemical, biological, or microbiological properties that must be within specified limits to ensure product quality. For liposomal drug products, key CQAs include particle size and size distribution, lamellarity, encapsulation efficiency, drug release rate, surface charge (zeta potential), and sterility. These attributes directly influence the

pharmacokinetics, biodistribution, and therapeutic efficacy of liposomal formulations. Various analytical techniques are employed for characterization, such as light scattering, electron microscopy, and chromatography [73]. The development of liposomal formulations faces challenges in ensuring batch-to-batch reproducibility and stability. To address these issues, the QbD approach has been proposed as a systematic, risk-based strategy for pharmaceutical development [74, 75]. This approach aims to establish relationships between critical material attributes, process parameters, and product quality attributes, ultimately defining a design space for optimal formulation [63]. Implementing comprehensive QC measures and adopting QbD principles can help overcome the complexities associated with liposome production and ensure high-quality pharmaceutical products.

The quality and production control of liposome-based drugs involve several critical stages, each with specific parameters to monitor [73]. Initially, the lipid film is produced and dispersed or degraded, during which key factors such as the amount of residual organic solvents in the lipid film, the integration rate of the API, liposome size post-dispersion, stability, and pH value are meticulously controlled. Following this, liposomes of the desired size are manufactured, non-integrated APIs are separated, and the formulation is sterilized via filtration. At this stage, the integrated API amount, liposome size, lipid component concentration, stability, and pH value remain under scrutiny. The final production stage often involves lyophilization, where parameters like residual moisture, stability, and the percentage of drug integration after rehydration are evaluated.

A critical aspect of liposome-based drug development involves assessing the behavior of the API in physiological environments. Reliable, validated *in vitro* methods are essential to evaluate API release, including tests simulating "leakage" under varied conditions like temperature and pH. Stability during storage, consistency across batches, and thorough reviews of production processes under intended usage conditions are equally important. Additional parameters include evaluating the integrity of the liposomal formulation in plasma, lipid bilayer phase transitions, surface charge, and the pH gradient of the liposomal inner chamber. For finished products, the encapsulated API percentage, stability of the API and lipids, release rate *in vitro*, and physical state of the API (e.g., precipitate formation for doxorubicin) must be determined. For conjugated formulations, attributes such as PEG quality, molecular weight, and distribution on the liposome surface are critical [76].

In case of surface-coated liposomes (as described in Section 3.2), since the coverage density and thickness of the layer affect cellular uptake and control nanoparticle transport through biological matrices, FDA guidelines recommend that the coating thickness of nanomaterials be detailed in the dossier [77]. The European Medicines Agency's reflection paper mentions that the influence of surface modifications on product stability, pharmacokinetics, biodistribution, biomolecular interactions, and receptor-mediated cellular interactions should be carefully considered [78]. Additionally, the coating material must be thoroughly characterized and controlled, including its consistency and reproducibility, surface coverage heterogeneity, orientation and conformational state of the ligand, physicochemical stability, premature detachment, and degradation of the coating. A comprehensive testing regimen

should be established for each batch, aligning with the parameters outlined above to ensure compliance with quality standards. Globally, regulatory bodies impose distinct requirements on the production, QC, and clinical evaluation of liposome-based drugs [76].

QC focuses on the evaluation and testing of raw materials, intermediates, and final products to ensure compliance with predefined specifications. Liposomal drug products undergo rigorous testing for parameters such as size distribution, zeta potential, drug encapsulation efficiency, and release profiles. QC testing often utilizes state-of-the-art analytical techniques, including DLS for particle size analysis, transmission electron microscopy (TEM) for structural characterization, and high-performance liquid chromatography (HPLC) for drug quantification. QC also includes microbiological testing to ensure sterility, especially for parenteral formulations. Batch-to-batch consistency is critical, and robust QC systems help detect deviations and initiate corrective actions promptly. Different methods used for characterization of liposomes are depicted in Figure 7.3.

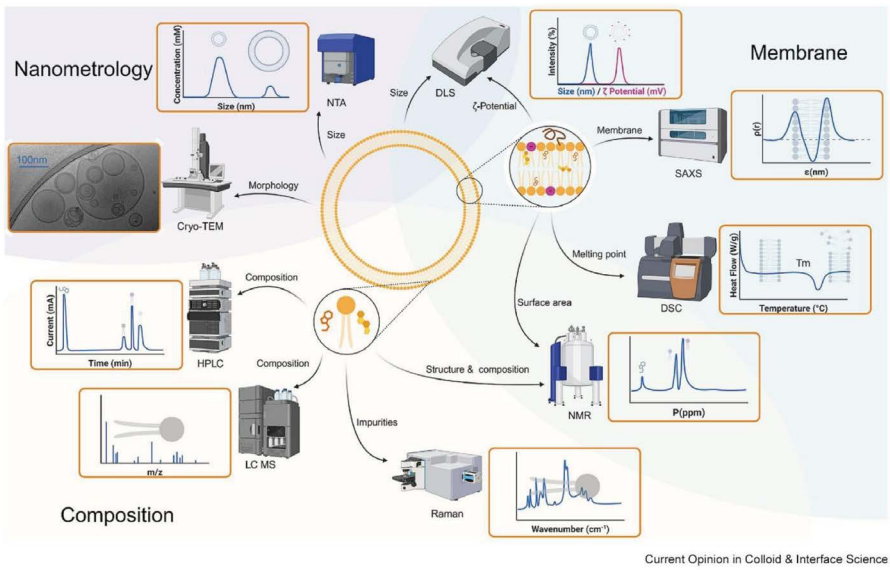


**FIGURE 7.3** Methods for liposome characterization. Particle size, distribution, and morphology are assessed using dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and cryo-transmission electron microscopy (Cryo-TEM). Membrane and surface characteristics are evaluated by DLS, small-angle X-ray scattering (SAXS), differential scanning calorimetry (DSC), and nuclear magnetic resonance (NMR). Chemical composition, drug loading, impurities, and structural information are obtained through high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), Raman spectroscopy, and NMR. (Reproduced from Liposomes: Bridging the gap from lab to pharmaceuticals by Eugster R and Luciani P, published in *Current Opinion in Colloid & Interface Science*, 2025, under the Creative Commons CC-BY license. Available at: https://doi.org/10.1016/J.COCIS.2024.101875 [7].)

The size, surface charge, and PDI of drug delivery systems are key attributes that significantly influence their internalization and targeting efficiency in biological environments [79, 80]. Among the commonly employed characterization methods, batch-mode techniques such as DLS are widely used due to their simplicity and speed. DLS provides estimates of size distribution and PDI, reflecting the uniformity of the nanosystem. When combined with electrophoretic mobility measurements, such as electrophoretic light scattering, it can also determine the surface charge based on zeta potential distribution. However, DLS has limitations, particularly in analyzing polydisperse systems, as its resolution is confined to a factor of three. This constraint can lead to challenges in distinguishing multimodal particle populations [81].

Microscopy-based methods, such as TEM, scanning electron microscopy (SEM), and atomic force microscopy (AFM), offer alternatives to DLS. These techniques circumvent the drawbacks of DLS by conducting particle-by-particle analysis from images, enabling precise characterization of individual nanoparticles. However, their broader application is constrained by the complexity of sample preparation, limited accessibility, and high costs [82].

Beyond physicochemical characterization, it is crucial to evaluate nanosystems within biologically relevant environments. Fluorescence-based labeling is a common approach for tracking nanosystems *in vitro* [83] and *in vivo* [84]. Additionally, fluorescence techniques have been adapted for physicochemical characterization, with size estimation through fluorescence microscopy [85] and flow cytometry [86]. These methods enable a direct correlation between physicochemical properties and behavioral characteristics. However, introducing fluorescent labels may alter the nanosystem's surface properties or result in label detachment [87]. Fluorescent signals also degrade over time and are unsuitable for long-term tracking, while intense illumination can induce phototoxicity in live samples.

Emerging label-free techniques aim to combine physicochemical and behavioral characterization without the need for markers. These include surface plasmon resonance (SPR) [88], nanoparticle tracking analysis (NTA) [89], coherent anti-Stokes Raman scattering (CARS) [90], and quantitative phase microscopy (QPM) [91].

### 7.2.7 Stability Studies

Stability studies are a cornerstone of QA for liposomal formulations, which are often prone to aggregation, drug leakage, and oxidation. Accelerated and long-term stability testing under ICH-recommended conditions (e.g., varying temperatures and humidity levels) evaluates the shelf-life of liposomal products. The inclusion of cryoprotectants during freeze-drying processes and antioxidants in formulations can enhance stability. Analytical methods such as differential scanning calorimetry (DSC) are employed to study lipid phase transitions, while spectroscopy techniques like Fourier-transform infrared (FTIR) are used to monitor chemical stability. Stability studies also assess the impact of storage containers, as interactions between liposomes and packaging materials can compromise product integrity [92, 93].

## 7.3 REGULATORY GUIDELINES (FDA, EMA)

The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have established stringent regulations for liposomal drugs, aligning with Good Manufacturing Practices (GMPs) and ICH guidelines. The FDA's guidance on liposome drug products, updated in 2018, focuses on the characterization of liposome size, drug encapsulation efficiency, and release kinetics. It also emphasizes the need for validated methods to ensure reproducibility across batches [94]. Similarly, the EMA requires liposome-based products to meet criteria for particle size distribution, surface charge, and stability under varied storage conditions [95]. These agencies highlight the importance of QbD, wherein CQAs are defined early in development, and manufacturing processes are designed to maintain these attributes throughout the product lifecycle.

Regulatory guidance for liposomal drug products has been developed by both the FDA and EMA to address the unique challenges of these complex delivery systems [96]. These guidelines cover critical aspects of liposome development, including characterization, manufacturing, and QC [76]. Key considerations include lipid excipients, manufacturing methods, nanosizing techniques, drug loading methods, and CQAs [76]. The FDA recommends determining CQAs that impact product performance and utilizing risk assessments to link structure-function relationships [97]. GMPs are required for nanomaterial-containing drugs, and existing ICH guidelines for nonclinical studies are generally applicable [97]. Drug release characteristics should be analyzed, and differences between liposomal and non-liposomal formulations should be included in applications [97].

## 7.4 CONCLUSIONS

Advancements in liposome manufacturing and QC have significantly enhanced their commercial viability. Ongoing innovations continue to expand their therapeutic applications, promising a robust future for liposome-based drug delivery systems.

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# 8 Production of Polymer-Based Nanoparticles

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## 8.1 INTRODUCTION

Nanoparticles are submicron-sized particles, that is, particles with average size in the range of 1–1000 nm. This is a drug delivery vehicle, highly investigated by scientists to fulfill one or combination of drug delivery goals like release rate modulation, protection from physiological environment, increase bioavailability. Nanonization is mostly targeted to increase dissolution rate of drug molecules, which are poorly soluble in water, that is, BCS class II and IV drugs. However, sometimes the drug molecule entrapped in polymer shows slow/sustained drug release. In such cases, polymeric microparticles are preferred to formulate sustained-release formulations, as they show more sustained release due to additional large size, along with the release-retardant polymer.

Polymeric nanomedicines are usually formulated to modify drug release pattern, either as polymer-drug conjugate for increased half-life and bioavailability, or for controlled release using degradable polymer (Bobo et al., 2016). To increase half-life, nanocapsules are generally coated with nonionic surfactants, to reduce immunological reaction, such as opsonization. There are several polymeric nanoparticles approved by USFDA and some nanoparticles are under clinical trial. Protein nanoparticles also use the same principle of using biocompatible natural proteins to carry the drug molecule. Paclitaxel nanoparticle approved for cancer treatment is albumin nanoparticle. Reduction of particle size below 200 nm is often carried out to protect the vehicle from being recognized by the body defense system. Reticuloendothelial defense system or defensive proteins generally cannot recognize nanoparticles as external body, hence they are protected from being metabolized. This mechanism is used to increase the mean residence time of drug or delivery system in the body, and hence can help in increasing patient compliance by reducing dosing frequency.

Nanoparticles are also used to improve bioavailability of drug molecule. One of the reason is reduced metabolism, as discussed above. Oral absorption of hydrophobic nanoparticles through Peyer's patches improves oral bioavailability of nanoparticles. This fact has been used for targeting lymphatic organs like lungs (e.g. Bachhav et al., 2018). Nanoparticles delivery through nasal route is also used to improve bioavailability of certain drugs in brain, due to their improved ability to cross blood–brain barrier (BBB). The global nanomedicine market is anticipated to reach USD 350.8 billion by 2025, according to a new report by Grand View Research, Inc. (Nanomedicine Market Size Worth $350.8 Billion By 2025 | CAGR: 11.2%. Available

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at: https://www.grandviewresearch.com/pressrelease/global-nanomedicine-market, 2017). The key players operating in this industry include Pfizer Inc., Ablynx NV, Nanotherapeutics Inc., Nanoviricides Inc., Abraxis Inc., Arrowhead Research Inc., Celgene Corporation, Bio-Gate AG, and Merck.

## 8.2 CLASSIFICATION OF NANOPARTICLES

Nanoparticles can be broadly classified based on its chemical composition:

1. Drug nanocrystal-based nanosuspension or nanoparticles
2. Natural polymer-based nanoparticles:
   i. Lipid-based nanoparticles
   ii. Protein and peptide nanoparticles
   iii. Polysaccharide-based nanoparticles
3. Synthetic polymeric and copolymeric nanoparticles: PLGA, polyacrylates.
4. Non-polymeric inorganic nanoparticles: carbon, metallic, silica based, etc.

Table 8.1 highlights polymers that are widely used in constituting nanoparticles designed as drug carriers.

### 8.2.1 Natural Polymer-Based Nanoparticles

#### 8.2.1.1 Lipid-Based Nanoparticles

Lipid nanoparticle (LNP) is a broad class of nanoparticle, which includes liposomes, solid LNP, nano lipid complexes, etc. These are more biocompatible, as lipids generally used are phospholipids and other solid lipids, which are natural composition of human body. LNP contributes to the major category of FDA-approved nanoparticles. Liposomes are lipid bilayers, which can be used to deliver both lipophilic and hydrophilic drugs. FDA-approved doxorubicin liposomes entrap the active molecule in the hydrophilic part, whereas amphotericin liposome carries the active molecule in the lipophilic part. Amphotericin lipid complexes and liposome are also approved drug delivery systems. Chapter 6 covers lipidic nanoparticles in detail.

#### 8.2.1.2 Protein- and Peptide-Based Nanoparticles

In the recent years, proteins are used as alternative to synthetic polymers for preparation of nanoparticles because of their biocompatibility and safety. Proteins, which are linear polymers of L-α-amino acids having a wide variety of structures and functions, show unique potential applications in biomedical and material science. The main advantageous feature is proteins are biocompatible and show biodegradability. Proteins are accessible to modifications for tailor drug-binding and binding imaging or targeting entities. They are amphiphilic in nature and hence considered as ideal materials for nanoparticle preparation by interacting well with both drugs and solvent. They have been successfully synthesized from various proteins including water-soluble (e.g., bovine and human serum albumin (HSA)) and water-insoluble proteins (e.g., zein and gliadin) (https://www.cd-bioparticles.net/protein-based-nanoparticles-

**TABLE 8.1**
**Most Widely Used Polymers Constituting Nanoparticles Designed as Drug Carriers**

| Material | Full Name | Abbreviation/Brand Names |
|---|---|---|
| Synthetic homopolymers | Poly(lactide) | PLA |
| | Poly(lactide-co-glycolide) | PLGA |
| | Poly(epsilon-caprolactone) | PCL |
| | Poly(isobutyl cyanoacrylate) | PICBA |
| | Poly(isohexyl cyanoacrylate) | PIHCA |
| | Poly(n-butyl cyanoacrylate) | PBCA |
| | Poly(acrylate) and poly(methacrylate) | Eudragit* |
| Natural polymers: Lipid, protein and polysaccharides | Chitosan | |
| | Alginate | |
| | Gelatin | |
| | Lecithin and cholesterol | |
| | Albumin | |
| Copolymers | Poly(lactide)-poly(ethylene glycol) | PLA-PEG |
| | Poly(lactide-co-glycolide)-poly(ethylene glycol) | PLGA-PEG |
| | Poly(epsilon-caprolactone)-poly(ethylene glycol) | PCL-PEG |
| | Poly(hexadecylcyanoacrylate-co-poly(ethylene glycol) cyanoacrylate) | Poly(HDCA-PEGCA) |
| Colloid stabilizers and surfactants | Dextran | |
| | Pluronic F68 | F68 |
| | Poly(vinyl alcohol) | PVA |
| | Copolymers (see above) | |
| | Polyoxyethylene sorbitan esters | Tween and Span* |

* brand names.
*Source:* Vauthier and Bouchemal (2009).

production?gad=1&gclid=EAIaIQobChMI_KubyNKuggMVLzCDAx0CuALTEAAYAiAAEgLJA_D_BwE on dated 06 Nov. 2023). Collagen is a natural protein, which is found in connective tissues. Collagen nanoparticles have applications in tissue engineering, wound healing, and drug delivery (https://www.cd-bioparticles.net/polymeric-nanoparticles-production?gad_source=1&gclid=EAIaIQobChMIt_6qq8e5ggMVDqJmAh1lHw8IEAAYASAAEgK7dvD_BwE accessed on 10 Nov. 2023). Table 8.2 describes advantages and drawbacks of different protein nanoparticles.

Protein nanoparticles are usually manufactured using proteins, such as albumin, gelatin, fibroins, gliadine, 30Kc19, lipoprotein, legumin, and ferritin proteins. These are prepared through emulsion, electrospray, and desolvation methods (Hong et al., 2020). One of the very successful commercial formulations is paclitaxel nanoparticle with the trade name Abraxane, which is an injectable nano suspension where Paclitaxel-albumin based protein-bound nanoparticles are available as injectable suspension. In it paclitaxel is formulated as albumin-bound nanoparticles with a mean particle size of approximately 130 nm (https://dailymed.nlm.nih.gov/

**TABLE 8.2**
**Advantages and Drawbacks of Different Protein Nanoparticles**

| Material | Advantages | Drawbacks | Remarks |
|---|---|---|---|
| Silk protein fibroin (main protein present in silk fibers, comprising 65% to 85% of the total protein) | High stability | Sericin may cause immunogenic reactions | Drug encapsulation and release profiles, fibroin crystallinity plays an important role, which is influenced by salt concentration, type and amount of organic solvent, and temperature range. |
| | Flexibility with high mechanical strength, suitable for various machining conditions | Slow degradation of silk II crystalline antiparallel β-sheet domains | Fibroin MW, crystallinity, encapsulated drug properties, and manufacturing conditions, may affect nanoparticle properties, like average size, size distribution, surface zeta potential, drug encapsulation, release profile, and stability of particle formation. |
| | Biodegradability | | The drug-loading dose depends on the pKa and the solubility of the captured drug. Drug molecules are primarily associated with fibroin through electrostatic interactions, hydrogen bonding, and/or hydrophobic interactions. |
| | Biocompatibility and low immunogenicity | | Since it is commonly extracted from silk produced by the *Bombyx mori* silkworm, by removing the external sericin via a thermochemical degumming process, so fibroin nanoparticles demonstrated non-cytotoxic as well as immunomodulatory properties via the inhibition of the activity of lipopolysaccharide-stimulated RAW 264.7 macrophages (i.e., nitrite production). |
| Human serum albumin (HSA) | High stability | Expensive cost | HSA is very stable against pH (stabilized in the pH range of 4–9), temperature (can be heated at 60°C for up to 10 hours) and organic solvents |
| | High-solubility HAS in physiological fluids | | HSA acts as a carrier for other substances to enhance the solubility of hydrophobic molecules in the blood. So, HSA is used to deliver various drug substances to specific tissues in the body. |
| | Biodegradability | | Biological origin |
| | Non-immunogenicity | | Human serum albumin (HSA) is a globular protein found mainly in the circulatory system composed of up to approximately 585 amino acids, which is equivalent to an MW of 66 kDa. |

*(Continued)*

**TABLE 8.2** *(Continued)*
**Advantages and Drawbacks of Different Protein Nanoparticles**

| Material | Advantages | Drawbacks | Remarks |
|---|---|---|---|
| | Non-toxic | | It can avoid unwanted interactions with serums that are often encountered after intravenous injection of complexes (Kratz, 2008). |
| | Availability and readiness | | HSA has a high affinity to various drugs, it is possible to effectively integrate these compounds using a matrix of HSA nanoparticles (Steinhauser et al., 2008; Ulbrich et al., 2009). Presence of various functional reactive groups, such as amino, thiol, and carboxyl, on the nanoparticle surface, facilitates covalent ligand binding and surface modification. |
| Gliadin (main proteins in wheat gluten and is extracted using 70% ethanol. | Biocompatibility | Large particle size | It is single-chain polypeptides from wheat gluten linked by intramolecular disulfide bonds, with each polypeptide chain having an average MW of 25–100 kDa |
| | Biodegradability | Rapid degradation speed | |
| | Non-immunogenicity | Gliadin nanoparticles are susceptible to pH, heating, and salt, thereby resulting in aggregation and instability (Joye et al., 2015) | Gliadin is rich in neutral and lipophilic amino acid residues. Neutral amino acids promote hydrogen bonding with the mucosa. The lipophilic amino acids interact with biological tissues through hydrophobic interactions. |
| | Non-toxic | | Water-insoluble proteins; so no additional curing step is required to maintain the integrity in water-based products. |
| | High stability | | Low solubility in aqueous solutions, except for extreme pH. This low water solubility is due to hydrophobic interactions and disulfide bonds which lead to a distinct folded structure of the protein. Because of this, gliadin nanoparticles can be used as a controlled-release system suitable for hydrophobic and amphiphilic drugs. |
| | Attaches to mucosa | | It is suitable for oral and local drug delivery systems. |

*(Continued)*

**TABLE 8.2** *(Continued)*
**Advantages and Drawbacks of Different Protein Nanoparticles**

| Material | Advantages | Drawbacks | Remarks |
|---|---|---|---|
| Gelatin (denatured protein gelatin is a natural water-soluble polymer that can be obtained by hydrolyzing collagen in alkaline or acidic mediators or by thermal or enzymatic degradation of collagen). | Biocompatibility | Low mechanical strength | It must be physically, biologically, or chemically crosslinked by various crosslinking agents to increase mechanical strength and lower the decomposition rate and solubility in aqueous solutions (Verma et al., 2018). Due to higher crosslinking density, the decomposition rate is slower. |
| | Biodegradability | Rapid degradation speed | Average size of gelatin is 20–220 kDa, and it is a soluble protein in water at above 35°C to 40°C. |
| | Ease of bridge and drug loading | | Commercially available gelatin is a heterogeneous mixture consisting of 18 specific amino acid polypeptide chains. The primary structure of gelatin enables various chemical modifications for instance drug attachment via its covalent bond. |
| | Safety and stability | | Cationic gelatin (type A with an IEP of 7–9) is derived from partial acid hydrolysis of pig skin type 1 collagen, while anionic gelatin (type B, IEP of 4.8–5) is extracted from alkaline bovine collagen. Gelatin contains hydrophobic groups, both cationic and anionic groups, and a three-helix structure with repeating sequences of glycine, proline, and alanine (Elzoghby, 2013; Sahoo et al., 2015). |
| | | | The three polypeptide α-chains in the helix structure enable high stability of gelatin. |
| Legumin (major storage proteins of soybean seeds (*Pisum sativum* L.) and it belongs to the 11S globulin protein family) | Bioadhesive | Low yield | Legumin has a molecular mass of 300–400 kDa. It is rich in sulfur-containing amino acids, and it consists of six subunits |
| | Wide surface area | | Coacervation method is frequently used for nanoparticle formation; The optimum pH for obtaining submicron-sized coacervates is near neutral. |
| | Small particle size | | The size of the particles ranges from 250–300 nm at pH 4.5–7. |

*(Continued)*

**TABLE 8.2** *(Continued)*
**Advantages and Drawbacks of Different Protein Nanoparticles**

| Material | Advantages | Drawbacks | Remarks |
|---|---|---|---|
| | Low immunogenicity | | Crosslinking with GA decreased the antigenic determinants of legumin, leading to the reduction in the immunogenicity of the protein. |
| | High stability | | The particles showed good stability when stored under neutral pH conditions. |
| 30Kc19 (Protein derived from silkworm hemolymph) | High stability | Low nanoparticle size and yield when using only 30Kc19α (When the nanoparticles were manufactured using only 30Kc19, the nanoparticles were not well formed and the size was too large. However, when 30Kc19-HSA nanoparticles were prepared with 50 wt% of 30Kc19 and HSA, the particle size was small and the drug activity was high.) | The 30K protein family (30Kc6, 30Kc12, 30Kc19, 30Kc21, 30Kc23) is a group of proteins derived from the hemolymph of silkworms (*Bombyx mori*) that have similar structures (Izumi et al., 1981). The molecular weight of the 30K proteins is about 30 kDa. |
| | Increased cell growth and viability | | It plays role in cell growth and viability in various cells, and has an enzyme-stabilizing effect (Park et al., 2003; Park et al., 2012). It has been shown that 30Kc19, the most abundant protein in the 30K protein family, has a cell-penetrating effect (Park et al., 2014). The 30Kc19 protein consists of six alpha-helixes of N-terminal domain and 12 beta-strands of the C-terminal domain (Park et al., 2017). |
| | Biodegradability<br>Non-immunogenicity<br>Non-toxic | | Protein nanoparticles were made via the desolvation method. At high pH and low concentration of 30Kc19α smaller nanoparticle size was achieved. |
| | Enzyme-stabilizing property | | The α-helix domain of the 30Kc19 protein (30Kc19α domain) is associated with the enzyme-stabilizing effect of the 30Kc193 protein on the cargo protein (Park et al., 2017). |
| | Cell-penetrating property | | The morphology of all 30Kc19 nanoparticles was spherical that showed higher cellular uptake in human fibroblasts, as compared with the HSA nanoparticles. |

*(Continued)*

**TABLE 8.2** *(Continued)*
**Advantages and Drawbacks of Different Protein Nanoparticles**

| Material | Advantages | Drawbacks | Remarks |
|---|---|---|---|
| Lipoprotein (lipoproteins are natural nanoparticles that transport fats within the body (Thaxton et al., 2016) | Non-immunogenicity<br>Biodegradability<br>Biocompatibility<br>Long circulation half-life<br>Naturally targeting property | Difficult to separate native LDL | Categorized according to size and density, ranging from high-density lipoprotein (HDL; 7–13 nm), low-density lipoprotein (LDL; 22–27 nm), intermediate-density lipoprotein (IDL; 27–30 nm), very low-density lipoprotein (VLDL; 35–80 nm), and chylomicrons (80–1200 nm) (Segrest et al., 1994). |
| Ferritin (ferritin, a protein with iron storage capability, is a hollow globular protein of MW of 474 kDa) | High stability | High cost | Two classes of ferritin (H and L) in mammalian cells, which function complementarily in relation to one another in the iron absorption process (Chasteen and Harrison, 1999). Twenty-four copies of the same ferritin subunits are self-assembled forming ferritin nanoparticles |
| | pH stability<br>Thermal stability | | A remarkable property of ferritin is that it is thermostable and can withstand high temperatures up to 75°C for 10 minutes and is also stable in the presence of denaturants. Protein structure disassembles at pH 2.5, but restoration is possible when returning to pH 7.5 |
| | Biodegradability | | It is found in microorganisms, plants, and animals; comprised of 24 subunits, in which a 6-nm inorganic core of hydrated iron oxide ferrihydrite is surrounded by a spherical polypeptide shell (Apoferritin) (Zeth et al., 2016). |

dailymed/drugInfo.cfm?setid=24d10449-2936-4cd3-b7db-a7683db721e4 accessed on 09 Nov. 2023). This NP formulation completely eliminates Cremophor EL and ethanol in Taxol, it is administered in a shorter period of 30 minutes with no need for premedication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3806207/ accessed on 09 Nov. 2023). Ontak (Eisai Inc) is another protein-based nanoparticles formulation for intravenous injection for the treatment of patients with persistent or recurrent cutaneous T-cell lymphoma (CTCL) whose malignant cells express the CD25 component of the interleukin (IL)-2 receptor (CD25+).

Mixing positively charged glatiramer acetate (GA) (the active substance of Teva's Copaxone drug, containing random polypeptides of four amino acids (alanine, glutamic

acid, tyrosine, and lysine)) and the negatively charged genetic material in correct proportions produces small, stable, and highly positively charged nanoparticles. This simple GA-pDNA formulation produced high levels of transfection efficiency in HeLa and A549 cells (lung and cervical cancer cells), but has low toxicity. GA-pDNA nanoparticles may be prepared by mixing a genetic payload with approved GA therapeutics (e.g., Copaxone), thus offering intriguing possibilities for translational gene therapy studies is one example of protein nanoparticles (Alhakamy and Berkland, 2019).

#### 8.2.1.3 Polysaccharide-Based Nanoparticles

Polysaccharides have attracted considerable attention in a broad range of applications in drug delivery in recent years. Polysaccharides have remarkable features such as biocompatibility, biodegradability, renewable origin, and have facile modification. Polysaccharide nanoparticles are promoted for their applications in various areas and particularly in biomedicine. Plucinski et al. (2021) reviewed and highlighted the properties of common polysaccharides used in nanoparticle formation as well as strategies to fabricate polysaccharide nanoparticles (Table 8.3).

Polysaccharides are natural polymers consisting of monosaccharides linked by glycosidic bonds. Chitosan, cellulose, starch, hyaluronic acid, and dextran are some of the commonly used polysaccharides. Similar to proteins and glycosaminoglycans, polysaccharides are extracted from various natural sources, for example, plants, microbes, and animals (Mizrahy and Peer, 2012). The activities and biological properties of different polysaccharides are diverse due to their different chemical structures and ionic nature. The functions and applications of polysaccharides are directly related to their chemical and biochemical specifications. Table 8.3 discusses the properties of common polysaccharides used in nanomedicine, cosmetics, food, etc.

**TABLE 8.3**
**Properties of Some Common Polysaccharides Used for Formulation of Nanoparticles**

| Polysaccharide | Solubility in Water | Molar Mass (kDa) | Source |
|---|---|---|---|
| Alginate | Sodium or potassium salt are water soluble | 32–400 | Bacteria and algae |
| Carrageenan | Soluble in hot water | 100–1000 | Seaweed |
| Chitin | Insoluble | 1000–2500 | Exoskeleton of non-mammals |
| Chitosan | Soluble under acidic condition | 100–500 | *N*-Deacetylation of chitin |
| Cellulose | Insoluble | 50–2000 | Higher plants |
| Dextran | Soluble | 3–2000 | Bacteria |
| Hyaluronic acid | Soluble | 20–10 | Mammals |
| Pullulan | Soluble | 100–250 | Bacteria |
| Starch | Insoluble in cold water | Amylose (~100) and amylopectin (1000–10,000) | Higher plants |

*Source:* https://pubs.rsc.org/en/content/articlehtml/2021/tb/d1tb00628b accessed on 20 Nov. 2023.

A number of methods have been proposed to prepare NPs based on natural polysaccharides. Proper synthesis method is selected based on the targeted application and its requirements. Many factors such as particle size, reproducibility of the release kinetic profiles, thermal and chemical stability of the active agent, and stability of the final product and proposed route of administration associated with the final product have to be taken into account. The native charges of polysaccharides can be exploited for the synthesis of polysaccharide NPs or covalent crosslinking can be implemented. Mizrahy and Peer (2012) discussed the main mechanisms during the synthesis of polysaccharide NPs in their review in detail, which are categorized as chemical (covalent) crosslinking, physical (ionic) crosslinking, polyelectrolyte-complexation and self-assembly. Due to the repeated number of monomer units on the polysaccharide a full network can be achieved. Hence, a complete gelation of the reaction medium has to be suppressed *via* the preparation conditions, for example, in dispersed systems (https://pubs.rsc.org/en/content/articlehtml/2021/tb/d1tb00628b accessed on 20 Nov. 2023).

In addition to the crosslinking process itself, the formation of NPs relies on the formation of materials on the nanoscale, that is, size-reduction using various preparative techniques. Some important and widely used methods are nanoprecipitation, complex coacervation, and emulsification. Also, the microfluidic technique has been proven to be a useful for the preparation of polysaccharide NPs, both while using emulsion and nanoprecipitation approaches (Chiesa et al., 2018). Other related methods developed by researchers are reviewed by Plucinski et al. (2021) (https://pubs.rsc.org/en/content/articlehtml/2021/tb/d1tb00628b accessed on 20 Nov. 2023). The relationship between the methods utilized for polysaccharide NPs synthesis and the properties (e.g., diameter) of the finished product is reviewed to showcase how the methods affect the final properties.

#### 8.2.1.4 Cyclodextrin-Based Macromolecular Complexes

Cyclodextrins (CDs) are cyclic oligosaccharides and amphiphilic compounds containing at least six D-(+) glucopyranose units, attached by α-(1, 4) glycosidic bonds (Kurkov and Loftsson, 2013). These are also known as cycloamyloses or cyclomaltodextrins. CDs are obtained from enzymatic degradation of starch from corn, potato, and other sources of plant origin. This was discovered by French scientist Villiers, who isolated crystalline compound called "cellulosine" in 1891 (Vikas et al., 2018). In the following decades, Austrian microbiologist Schardinger later demonstrated the role of glycosyltransferase from *Bacillus macerans* in production of CDs from starch. Naturally occurring CDs-alpha, beta, and gamma, were identified which are referred to as Schardinger sugars. Schardinger also identified β-CD as "cellulosine" depicted by Villiers (Loftsson and Duchene, 2007). Nowadays, CDs are exploited for various applications in food, pharma, cosmetic, biotech, and textile industry (Hashimoto, 2002; Rogers, 2016).

CDs have been extensively used for the solubility enhancement of poorly water-soluble drugs. CDs can be used for the preparation of nanoparticles for improving drug loading in nanosystem. These nanomedicines help in improving stability, site-specific/targeting, improving solubility profile, with consequent improvement in bioavailability, with the possibility of controlled release, and improving safety and efficacy. Cyclodextrins were also found suitable for simultaneous diagnosis and therapeutics for better treatment procedures (Cyclodextrin-Based Nanoparticles for Drug Delivery

and Theranostics – https://pmc.ncbi.nlm.nih.gov/articles/PMC7191229/ accessed on 23 Nov. 2023). Modification in CDs can play a vital role in improving the performance of NP formulation. The natural CDs and their derivatives such as HP-β-CD and HP-γ-CD complexes or self-assemble in nanoscale aggregates in aqueous solutions (Sharma and Baldi, 2016). *In vitro* characteristics of CDs show impact on the properties of NPs including particle size, drug loading, and release from formulation (Bartlett and Davis, 2007; Zhang et al., 2010) and stability of formulation (Baek et al., 2016).

### 8.2.2 Synthetic Polymer-based Nanoparticles

Polymeric nanoparticles can be manipulated and have the potential of reabsorption in the body. They have favorable properties such as good biocompatibility, easy design, and preparation. They form a variety of structures and interesting bio-mimetic characters, and especially have the ability to deliver therapeutic agents directly into the intended site of action with superior efficacy. They have attracted greater attention in the last few decades as a multifunctional nanotechnological drug delivery system. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed, or chemically attached (https://www.cd-bioparticles.net/polymeric-nanoparticles-production?gad_source=1&gclid=EAIaIQobChMIt_6qq8e5ggMVDqJmAh1lHw8IEAAYASAAEgK7dvD_BwE accessed on 10 Nov. 2023). With its characters as nanocarriers, polymeric nanoparticles protect drugs against *in vitro* and *in vivo* degradation, release the entrapped molecule in a controlled manner, and also offer the possibility of drug targeting to achieve increase in drug therapeutic efficacy and reduction in side effects. Polymeric nanocarriers have numerous properties like simple to synthesize, inexpensive, biocompatible, biodegradable, non-immunogenic, non-toxic, and water soluble. Polymeric carriers avoid the interaction of the drug with macromolecules such as proteins, which could sequester the active ingredients preventing its arrival at the action place. Polymer-based systems are more cost-effective and easier to produce and scale up than liposomes with long stability profiles. Advantages of this type of carrier are decreased toxicity, adverse drug reactions, and better drug utilization. Chitosan, poly(methyl methacrylate), polyhydroxyalkanoates, poly-(lactic-co-glycolic acid) are typical polymers which are most widely used in drug delivery system and show great potential in therapeutic diagnostics applications. The currently marketed nanoparticles based on synthetic polymers are provided in Table 8.4.

**Classified by polymer composition** (https://www.cd-bioparticles.net/polymeric-nanoparticles-production?gad_source=1&gclid=EAIaIQobChMIt_6qq8e5ggMVDqJmAh1lHw8IEAAYASAAEgK7dvD_BwE accessed on 10 Nov. 2023):

- **Methacrylate:** Polymer nanoparticles derived from methacrylate monomers. They are used in various applications like dental materials, drug delivery, and tissue engineering.
- **Polyhydroxyalkanoates (PHA):** Biodegradable polymers produced by microorganisms. PHA nanoparticles are used in packaging, agricultural films, and controlled drug delivery.

**TABLE 8.4**
**Approved Marketed Drug-Loaded Polymer-Based Nanoparticles**

| S.No. | Drug Loaded | Type of Nanoparticle | Trade Name (Company Name) | Applications | Approval Date |
|---|---|---|---|---|---|
| 1 | Adenosine deaminase enzyme | PEGylated | Adagen®/ pegademase bovine (Sigma-Tau Pharmaceuticals) | Severe combined immunodeficiency disease (SCID) | 1990 |
| 2 | Pegaspargase | Polymer–protein conjugate PEGylated L-asparaginase | Oncaspar (Sigma Tau) | Acute lymphocytic blood clot | 1994 |
| 3 | Glatiramer acetate | Random copolymer of L-glutamate, L-alanine, L-lysine and L-tyrosine | Copaxone (Teva) | Multiple sclerosis | 1996 |
| 4 | Docetaxel | Micelle | Taxotere (Sanofi Aventis) | Antineoplastic | 1996 |
| 5 | Verapamil HCl | PLGA nanoparticles | Verelan PM (Schwarz Pharma) | Hypertension, angina, and rhythm disorders | 1998 |
| 6 | Sevelamer hydrochloride | Poly(allylamine hydrochloride) | Renagel® (Sanofi) | Chronic kidney disease, increase circulation and therapeutic delivery | 2000 |
| 7 | Peginterferon alfa-2B | PEGylated IFN alpha-2b protein | PegIntron (Schering) | Hepatitis C | 2001 |
| 8 | Leuprolide acetate | Leuprolide acetate and polymer [PLGH (poly (D,L-lactide-coglycolide)] | Eligard (Tolmar) | Prostate cancer | 2002 |
| 9 | Ibritumomab tiuxetan | Suspension | Zevalin (Spectrum Pharms) | Lymphoma | 2002 |
| 10 | Pegfilgrastim | PEGylated GCSF protein | Neulasta (Amgen) | Leukopenia by chemotherapy | 2002 |
| 11 | Peginterferon alfa-2A | PEGylated IFN alpha-2a protein | Pegasys (Hoffman-La Roche) | Hepatitis B and C | 2002 |
| 12 | Pegvisomant | PEGylated HGH receptor antagonist | Somavert (Pharmacia) | Acromegaly | 2003 |

*(Continued)*

**TABLE 8.4** *(Continued)*
**Approved Marketed Drug-Loaded Polymer-Based Nanoparticles**

| S.No. | Drug Loaded | Type of Nanoparticle | Trade Name (Company Name) | Applications | Approval Date |
|---|---|---|---|---|---|
| 13 | Anti-VEGF aptamer | PEGylated (vascular endothelial growth factor) aptamer | Macugen®/ Pegaptanib (Bausch & Lomb) | Macular degeneration, neovascular age-related | 2004 |
| 14 | Chemically synthesized ESA (erythropoiesis-stimulating agent) | Methoxy polyethylene glycol-epoetin beta | Mircera®/ (Hoffman-La Roche) | Anemia associated with chronic kidney disease | 2007 |
| 15 | Methoxy PEG glycol-epoetin β | Chemically synthesized ESA (erythropoiesis-stimulating agent) | Mircera (Hoffman-LaRoche) | Anemia with chronic renal failure | 2007 |
| 16 | Certolizumab pegol | PEGylated antibody fragment (certolizumab) | Cimzia (UCB) | Crohn's disease<br>Rheumatoid arthritis<br>Psoriatic Arthritis<br>Ankylosing Spondylitis | 2008<br>2009<br>2013<br>2013 |
| 17 | Pegloticase | Polymer–protein conjugate (PEGylated porcine-like uricase) | Krystexxa (Horizon) | Chronic gout | 2010 |
| 18 | Trastuzumab | Maytansine derivative, DM1 | Kadcyla | Breast cancer | 2013 |
| 19 | Peginterferon beta-1A | Polymer–protein conjugate (PEGylated IFN beta-1a) | Plegridy (Biogen) | Multiple sclerosis | 2014 |
| 20 | PEGylated factor VIII | Polymer–protein conjugate (PEGylated factor VIII) | Adynovate (Baxalta) | Hemophilia | 2015 |
| 21 | Factor IX | Glycopegylated coagulation factor IX | Rebinyn (Novo Nordisk Inc.) | Hemophilia | 2017 |
| 22 | Triamcinoloneacetonide | PLGA hydrogel | Zilretta (Flexion Theraps Inc) | Osteoarthritis | 2017 |

*Source:* https://www.degruyter.com/document/doi/10.1515/ntrev-2021-0115/html?lang=en accessed on 8 Nov. 2023 and https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-018-0392-8/tables/2 accessed on 29 Dec. 2023.

- **Polylactic and poly(lactic-co-glycolic acid) (PLGA):** Biodegradable and biocompatible polymers used extensively in drug delivery systems due to their tunable properties and controlled release capabilities.
- **Polystyrene:** A synthetic polymer used in nanoparticle formulation due to its stability and ease of modification.

### 8.2.3 Non-Polymeric Inorganic Nanoparticles: Carbon, Metallic, Silica–Based, etc.

Inorganic nanoparticles are most often explored for their theranostic potential, that is, combined diagnosis and treatment (https://www.sciencedirect.com/science/article/pii/S221133982200079X accessed on 08 Nov. 2023). This functionality is difficult to achieve with lipid-based or polymeric nanoparticles. The most commonly used inorganic nanoparticles for biomedical applications include pure metals (especially plasmonic nanoparticles such as Au and Ag), metal oxides (e.g., solid or mesoporous $SiO_2$, $\gamma$-$Fe_2O_3$/$Fe_3O_4$), semiconductor materials (quantum dots), and calcium phosphates. Gold nanoparticles are extensively studied due to their high biocompatibility, ease of synthesis, and bioconjugation, as well as tunable optical and thermal properties. Further details on inorganic nanoparticles are available in Chapter 10.

## 8.3 PRINCIPLES OF MANUFACTURING NANOPARTICLES

Nanoparticles manufacturing is dependent on its chemical composition and spatial structure required to get due to different method of nanoparticles production (https://www.cd-bioparticles.net/polymeric-nanoparticles-production?gad_source=1&gclid=EAIaIQobChMIt_6qq8e5ggMVDqJmAh1lHw8IEAAYASAAEgK7dvD_BwE accessed on 10 Nov. 2023) like polymer micelles (PMs), which are self-assembled nanoparticles with a hydrophobic core and hydrophilic shell, used for delivering hydrophobic drugs. Polymer nanogels, which are crosslinked polymer networks that can swell and hold water, used for drug delivery and tissue engineering. Nanoemulsions, which are colloidal systems of oil and water stabilized by an emulsifying agent, used for drug delivery and food technology. Or dendrimers, which are highly branched nanoparticles with a well-defined structure, used for drug delivery and diagnostic imaging.

1. Agglomeration-based nanoparticles and nanosphere
   i. Polymeric agglomerates
   ii. Metallic agglomerates
2. Polymeric amphiphilic self-assembled micellar systems based nanoparticles:
   i. Emulsion based
   ii. Microemulsion or swollen micellar systems
   iii. Nanoemulsion
   iv. Polymeric micelles (PMs)
   v. Vesicular system: Liposomes and lipid complex
3. Poly-ionic complex or nanogels

4. Macromolecular complex-based nanoparticles: Drug-polymer complex/conjugates
   i. Dendrimeric complexes
   ii. CD-based complexes
   iii. Solid dispersion
   iv. Carbon nanotubes

Nanoparticles are manufactured by either bottom-up technique or top-down techniques or combination methodologies. Bottom-up techniques involve controlled precipitation/crystallization. It is basically precipitation from solution. Top-down technology involves size reduction from macro size to nanosize. In both the techniques, high shear energy is required. In bottom-up technique shear energy is needed to break liquid into small globules and in top-down the shear energy is needed for breaking large solid particles. Combination methodologies comprising of bottom-up and top-down methods is practically used for production. Several technologies and devices can be used for the production of polymeric nanoparticles via emulsion- or nanoprecipitation-based methods (Qi et al., 2018; de Castro et al., 2020). Table 8.5 compares different methods used for nanoparticles production.

## 8.3 Agglomeration-Based Nanoparticles and Nanosphere

These systems commonly include polymeric agglomerates. Typically, nanoparticles are precipitated in crystalline or amorphous form from a supersaturated solution (aqueous/organic) or emulsion/dispersion (Al-Kassas et al., 2017), by evaporation of dispersion medium or by use of a non-solvent or antisolvent (Salazar et al., 2012; Sinha et al., 2013). The bottom-up approach is broadly classified into two categories namely, precipitation methods, evaporation methods and self-assembling PMs or nanoparticle formation. Bottom-up technique can be carried out by either of the following procedures:

### 8.3.2 Crystallization and Precipitation Methods

#### 8.3.2.1 Nanoprecipitation

Nanoprecipitation technique was first introduced by Fessi et al. (1989) for the preparation of polymeric nanoparticles. The formation of particles is based on the precipitation and subsequent solidification of the polymers due to the interfacial deposition of polymer after displacement of semi polar solvents miscible with water, from a lipophilic solution. Briefly, drug and polymer are dissolved in a water-miscible organic solvent and added to the aqueous phase containing stabilizer under stirring. The decrease in interfacial tension between the aqueous and organic phase results in the rapid diffusion of organic solvent into aqueous phase. Small droplets of nanoparticles with a well-defined size characterized by a narrow distribution are formed instantaneously during the solvent flow, diffusion and surface tension at the interface of organic solvent and the aqueous phase causing turbulence. The nanosuspension produced is freeze dried using 5% mannitol as a cryoprotectant to obtain a fine powder of nanoparticles (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4352224/ accessed on 11 Nov. 2023; Rao and Geckeler, 2011).

**TABLE 8.5**
**Comparison of Different Nanoparticle Preparation Methods**

| Method | Advantage | Disadvantage |
|---|---|---|
| Colloidal milling | Well-characterized emulsions are produced having uniform size, It is easy to scale-up | High energy is required for emulsification |
| Emulsion/ solvent extraction | High stability | Generates particles larger than those obtained by desolvation |
| | Reaction conditions controls shape and size of nanoparticles | Thermodynamic instability → Need surfactants and stabilizers |
| | Encapsulation efficiency is high | |
| Emulsification–solvent evaporation | Used to encapsulate both hydrophilic and lipophilic drugs | Coalescence of nanodroplets is possible during evaporation process |
| Emulsification–solvent diffusion | It is feasible to control nanoparticle size | High volume of water is required |
| | Process is scalable | In the aqueous external phase, possibility of leakage of water-soluble drug |
| Emulsification–reverse salting-out | Minimum stress to fragile drugs | Incompatibility between the salts and the drugs is possible |
| | High loading efficiency | Purification is required to remove electrolytes |
| | Easy to scale-up | |
| Gelation of the emulsion droplets | Natural macromolecules, hydrophilic and biocompatible are used | Possibility to encapsulation of hydrophilic drugs only |
| Alkyl cyanoacrylates polymerization | Core-shell tuned nanoparticles are obtained with ease | Reaction for radical emulsion polymerization |
| | Nanoparticles size can be controlled by using surfactant | Purification is required |
| Interfacial polycondensation | Surfactants of lower concentrations used | Used for encapsulation of lipophilic drugs only, need purification |
| | By varying the monomer concentration, Nanocapsule thickness can be modulated | |
| Polymeric nanoprecipitation | Highly simple, reproducible and fast | In the organic phase there is low polymer concentration |
| | Low concentration of surfactant is required | |
| | Scalable | |
| Polyelectrolyte complex formation | Achieved easily | Required to optimize the positively and negatively charged molecule ratio |
| | Based on the nature of polyelectrolyte used in excess positively or negatively charged nanoparticles can be synthesized | |
| Complex coacervation of polyelectrolyte | Stability is high | Scale-up is difficult |
| | Nanoparticles are Smaller | |
| | Drugs (protein or peptide) which are sensitive can be delivered | |
| | Nanoparticle's shape and size of can be controlled by reaction conditions | |
| Nanoparticles from neutral nanogels | Organic solvent is not needed | Not for hydrophilic drugs |
| | Drug release can be controlled | |

*(Continued)*

**TABLE 8.5** ***(Continued)***
**Comparison of Different Nanoparticle Preparation Methods**

| Method | Advantage | Disadvantage |
|---|---|---|
| Ionic gelation | One step organic solvent free method | Particle disintegration by Weak Ionic interactions |
| | Drug encapsulation and release from the nanoparticle is controlled by pH or ion concentration stimulus | |
| Electrospray technique | High stability | Low flow |
| | Small nanoparticles | May induce degradation of some macromolecule due to the stress involved in the operation parameters (e.g., in drying thermal and shear stress happens in the nozzle) |
| | Can be scaled up at industry-level also it is already in use | |
| Nano spray-drying | Particle size, shape, and morphology can be controlled | Only for small-scale production |
| | One-step semi-continuous process | hydrophobic drugs incorporation is challenging |
| | Heat-sensitive substances can be processed with low risk of degradation | |
| | Cost-effective | |
| Desolvation method | Stability is high | For proteins that is minimally affected by the de-soluble process itself or by dilution by transporter proteins |
| | Manufacturing is simple | |
| | Nanoparticles are small | |
| | Encapsulation efficiency is high | |
| | Nanoparticle's shape and size of is controlled by reaction conditions | |
| Self-assembly | Encapsulation efficiency is high | Controlling the size and shape of nanoparticles is difficult |
| | Nanoparticles are smaller | Protein strain potential exists |
| | Stability is high | |

*Source:* Vauthier and Bouchemal (2009).

Usually, surfactants can be included in the manufacturing process to impart stability to the colloidal suspension, although their presence is not required to ensure the formation of nanoparticles or size reduction. The obtained nanoparticles are typically characterized by a well-defined size and narrow size distribution, which are better than those produced by the emulsification solvent evaporation procedure. Nanoprecipitation is a method frequently used for the preparation of polymeric NPs with approximately 170 nm dimensions. This method can be used for the preparation of both nanospheres and nanocapsules. Nanospheres are nanoparticles, in which the active molecule is dissolved or dispersed in the polymeric matrix. Nanocapsules are obtained when the active

molecule is entrapped in a shell of polymer. Manufacturing of nanocapsules generally requires dissolution of the molecule to be entrapped in water, which is then emulsified in the organic polymeric solution in organic solvent, then this emulsion is further dispersed in the external phase to form multiple emulsion, followed be evaporation of organic solvent (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464532/ accessed on 11 Nov. 2023). Opposite phases may be used for entrapment of water-insoluble drugs.

**Process variable of precipitation (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6967137/ accessed on 25 Nov. 2023):**

The process variables are temperature, rate of stirring, and infusion rate of solute solution into the antisolvent. These were streamlined in terms of how these influence the local supersaturation that is achieved at the initial phases of precipitation. The influence of processing parameters (volume and temperature of the heated aqueous solution; type of nozzle) and formulation aspects (total solid concentrations; stabilizer concentrations) on the size of suspended particles, can be determined by laser light diffraction. But, the size distribution of dispersed nanoparticles was shown to be mostly independent across the different formulation and processing parameters (Bosselmann et al., 2012; Soliman et al., 2017). It was found that the kind of stabilizer used in the heated aqueous solution is much more important than its temperature as an increase in temperature either resulted in an increase or decrease in the mean particle size. Processing also does not always require a customized atomizing nozzle to produce submicron- or micron-particles.

### 8.3.2.2 Polymer Precipitation Methods

In these techniques, polymer is precipitated with formation of either nanospheres or nanocapsules. These are easy and speedy production techniques with comparatively uniform size distribution. The mild solvents like ethanol, butanol, or acetone may be selected for these techniques to avoid toxic residues generated from solvents like methylene chloride and chloroform, etc. Nonsurfactant NPs can be prepared in the presence of CDs which can avoid complications associated with surfactants. Based on sequence and of addition of drug and polymer, these preparation techniques can be divided as follows (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7191229/ accessed on 23 Nov. 2023):

#### *8.3.2.2.1 Solvent Injection Method*

Hydrophobic components are dissolved in water-miscible organic solvent while hydrophilic components are dissolved in the aqueous phase. Organic phase injected into the aqueous phase, diffusion of organic phase into later phase leads to NPs formation (Qiu et al., 2015). This method has an advantage that the based on the nature of drug and its solubility, it can be dissolved in either aqueous phase or organic phase.

#### *8.3.2.2.2 Solvent Evaporation Method*

In this method, a CD is dispersed in an organic solvent. Drug solution in the same solvent added to a CD dispersion. Then aqueous phase is added to this mixture and an organic solvent is evaporated to obtain nanospheres or nanocapsules, which will be further freeze dried (Duchene et al., 2016).

#### *8.3.2.2.3 Solvent Diffusion*

The solution of a drug in ethanol is slowly added to aqueous phase with a peristaltic pump with continuous stirring to form nanocrystals (NCs). The nanocrystal dispersion is clarified by centrifugation in the refrigerated condition which is then freeze dried (Miyazaki et al., 2017).

#### *8.3.2.2.4 Inotropic Gelation Method*

Sodium carboxy methyl β-CD (SCM- β-CD) or its mixture with pentasodium tripolyphosphate (TPP) in aqueous form was added to chitosan (CS) solution under magnetic stirring. The positively charged CS and negatively charged SCM-β-CD and/or TPP spontaneously react via inotropic gelation to form NPs (Pant and Negi, 2018).

#### *8.3.2.2.5 High-Gravity Antisolvent Precipitation*

Another method of nanoparticle preparation is by polymer precipitation using high-gravity antisolvent for precipitation. In this method the drug and polymer are dissolved in a solvent and is mixed with antisolvent in a rotating packed bed chamber. The mixture is subjected to centrifugal force. This allows the mixture to pass through the packing, due to high gravity, thereby leading to the formation of uniform-sized nanoparticles (Sinha et al., 2013).

### 8.3.2.3 Crystallization of Supersaturated Solution

Fast cooling or congealing of supersaturated solution in organic solvent can help in controlling the particle size. This principle has been used and patented by Cipla Pharma in the size reduction of Paliperidone palmitate to achieve particles of size range below 2 micron to match the drug release profile of Invega Sustenna (Malhotra, Geena; Singh, Sarabjit; Ansari, Khalid Akhtar, Paliperidone palmitate particles and compositions thereof, WO2016199170A2, 2016).

A process is designed to increase the rate of crystal growth by nucleation simultaneously, reduce their growth rate in three phases: Seeding, ultrasonication and precipitation. In the precipitation phase, a highly concentrated solution of the compound is injected into a high volume of antisolvent, which leads to formation of amorphous nanoaggregates. Drug crystal seed are incorporated to the suspension. Then least amount of the solvent is added to give the least freedom to molecules that are mandatory for crystallization. To avoid the unnecessary growth of branches on the seeds, and to quicken their separation, mechanical stress, like ultrasonic waves, is introduced to the suspension. The suspension is sonicated for a couple of minutes to build its dissolution and crystal nucleation on the seeds and detachment of crystal from the seeds, called sonoprecipitation. This approach works on the cavitation principle to produce uniformly dispersed nanoparticles. The size of the final product depends on the frequency of ultrasound, duration, intensity, and horn length.

### 8.3.2.4 Supercritical $CO_2$ to Produce Nanoparticles

Use of supercritical gases is another approach to reduce the particle size of drugs. Dissolving the active molecule and polymer in supercritical $CO_2$ and then exposing this solution into a low-pressure area using a narrow nozzle orifice (<100 μm) results in the formation of nanosized drug particles. This process is known as the

rapid expansion of supercritical solution (RESS). Additional supercritical $CO_2$-based processes include the supercritical antisolvent (SAS) and the gas antisolvent (GAS) processes, which use supercritical $CO_2$ as an antisolvent, where the drug and polymer are previously dissolved in a solvent (Rodrigues et al., 2009, 2011; Padrela et al., 2017, 2018). The particle size of the drug precipitated depends on solvent/antisolvent ratio, drug concentration, degree of mixing, and rate of antisolvent addition (Reverchon, 1999; Reverchon et al., 2007). In addition, supercritical-assisted spray-drying (SASD) process has also been successfully used both to generate nanoparticles and control the polymorphic form. Supercritical cystallization for nanoparticle crystallization is carried out by two different methods, one is rapid expansion in supercritical solutions (RESS) and the second one is spray flash evaporation (SFE).

#### *8.3.2.4.1 Rapid Expansion in Supercritical Solutions (RESS)*

RESS can create nano-organic materials, and nanosized drugs by crystallizing them in a reasonable supercritical fluid, which is high-pressure carbon dioxide and high temperature. This is accomplished in a continuous way (Spitzer et al., 2017). These strategies can constantly produce well-dried formulation, because of the low solvency of the materials in carbon dioxide. But, the production amount limit is low, barely achieving production limits more than 1 gram/hour. In order to avoid each of these restrictions, another technique, the spray flash method was developed.

#### *8.3.2.4.2 Spray Flash Evaporation*

In the SFE system, ultrasound splash pyrolysis method was developed. The compound to be nanocrystallized, unadulterated one or blend of mixes, is broken down in a low boiling organic/natural solvent like ethanol and acetone. In these solvents, the solubility of most of the compound is in the range of 1 and 10 wt% at room temperatures. At that point the mixture is pressurized to ~40 bars, by compressed nitrogen gas. This is forced through a warmed empty cone nozzle into a primary vacuum chamber. The nozzle is warmed at temperatures in between 130°C and 160°C, while the evaporation chamber is set under vacuum at 520 mbar pressure. The extraordinary pressure drops from the high-pressure zone to the low-pressure zone, with ensuing temperature drop initiating evaporation instantaneously. This causes crystallization of the particles that was dissolved in the original solution. After crystallization, the crystals can be trapped in a cyclone, in an electrical precipitator or in filter (Spitzer et al., 2017).

### 8.3.3 Evaporative Methods

#### 8.3.3.1 Spray-Drying Is a Well-Known Evaporative Method

The nano spray-drying method is used to produce nanoparticles of pharmaceutical drugs. Buchi B-90, was developed by Buchi Labortechnik AG, Switzerland to produce nanoparticles of pharmaceutical drugs have been successfully produced using the nano spray-dryer. Onoue et al. (2012) prepared nanoemulsified particles with a mean diameter of 317 nm, which exhibited a 4500-fold increase in the dissolution rate of cyclosporine A. Nano spray-drying (Figure 8.1a) is a technique that is used for processing nanoparticles in liquid samples. Liquid samples are sprayed into

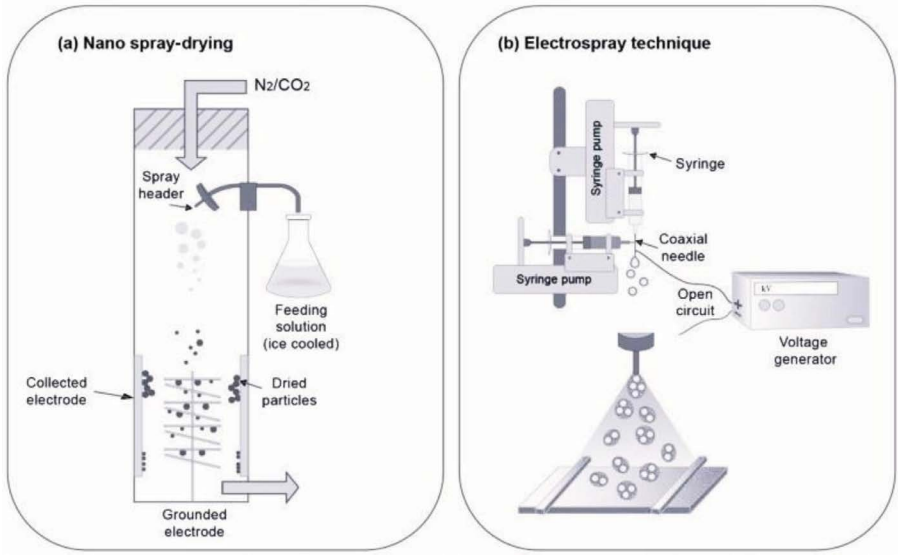



**FIGURE 8.1** (a) Nano spray-drying is the process of releasing and drying a protein liquid jet stream with a nozzle using heated nitrogen and carbon dioxide gas to produce nanoparticles. (b) The electrospray technique generates nanoparticles by ejecting a liquid jet stream through a nozzle that forms an aerosolized droplet by applying a high voltage to a protein solution supplied through a nebulizer. (Adapted from https://pmc.ncbi.nlm.nih.gov/articles/PMC7407889/figure/pharmaceutics-12-00604-f004/ accessed on 8 Nov. 2023.)

chambers containing heated nitrogen and carbon dioxide gas flow in the direction of spray from the nozzle (Oliveira et al., 2013). An electrode is at the bottom of the chamber to collect nanoparticles. The sprayed droplets are charged electrostatically by these electrodes, as they move toward the bottom of the chamber. This is step-by-step process, as well as a fast and cost-effective way of producing small-scale protein particles. This is used in drug delivery systems, where hydrophilic drugs are encapsulated in these spray-dried nanoparticles. The nanoparticles enable use of the technique on heat-sensitive specimens as solvent evaporation helps maintain the temperature of nanoparticle droplets (Haggag and Faheem, 2015).

#### 8.3.3.2 Spray Freeze-Drying

Another evaporative method to produce nanoparticles, where the drug solution is atomized into the cryogenic liquid resulting in the freezing of formed particles which are later lyophilized. This process generally produces amorphous nanoparticles with high surface area (Singh and Van den Mooter, 2016). Hu et al. produced 100-nm amorphous particles of danazol using a spray-freezing approach which exhibited high surface area and solubility (Hu et al., 2004).

#### 8.3.3.3 Electrospraying

Electrospraying is a method of liquid atomization. It has been used to manipulate materials into the submicron scale. This method requires the application of high

voltage to the protein solution to be able to spray the liquid jet stream through the nozzle for the formation of the aerosolized droplet (Wu et al., 2009; Gulfam et al., 2012). Aerosolized droplets contain collected colloidal-sized protein nanoparticles (Figure 8.1b). Using this method, drugs and nucleic acids can easily be integrated into nanoparticles with high efficacy. Solid particles can be produced via solvent evaporation (Champion et al., 2007). The needle gauge diameter, applied voltage, flow rate, and operating distance vary depending on the type of drug delivery system. The principle of electrospraying is to apply high voltage to polymer solutions to ensure that polymers come out of syringes in the form of nanoparticles (Dorozhkin, 2010; Bock et al., 2011). Indeed, electrospraying is a technology similar to electrical radiation used in the production of nanostructures. The advantages of electrospraying include cost-effectiveness, reproducibility, and high encapsulation efficiency (EE). Another advantage is the ease for the synthesis and stable protein or carbohydrate polymer nanoparticles without a decrease in EE and issues related with biocompatibility. The new version of the conventional electrospraying is the coaxial electrospraying method, which uses a coaxial spray head to simultaneously guide both solutions to the electric field. Yang et al. generated gliadin nanoparticles containing meletin using the electrospraying method. The particle size was 570 ± 80 nm, while the drug release efficiency was 28.8% within 1 hour of the treatment and 93.7% was released within 16 hours (Yang et al., 2018).

## 8.4 COMBINATION METHODOLOGIES AND ALTERNATE APPROACHES

### 8.4.1 Emulsification Methods

#### 8.4.1.1 Emulsification–Solvent Diffusion and Evaporation Method

This method is commonly applied to polymer nanoparticles but it has also been also used to produce protein nanoparticles. In its simplest form, a polymer solution (in organic solvent [O]) or protein solution (in aqueous buffer [W]) is dispersed using mechanical agitation or sonication to form an emulsion system (O/W or W/O), followed by the solvent/non-solvent being removed to form nanoparticles. In this method a partially water-miscible organic solvent or solvent mixture is used. After emulsification in aqueous phase, gradual diffusion of solvent in aqueous dispersion medium causes precipitation (Guinebretière et al. 2002; Limayem et al., 2004). This method is easy to scale-up and lead to more control on the size of nanoparticles. However, high volumes of water is to be eliminated and leakage of water-soluble drug into the saturated-aqueous external phase. The use of certain organic solvents, such as ethyl-acetate and chloroform, as well as surfactants, such as poly (vinyl alcohol) and polysorbate-80, is undesirable because it can alter the biological activity of protein therapeutics, thereby possibly resulting in unwanted reactions (Raghuvanshi et al., 1998; Sturesson and Carlfors, 2000). There is possibility to encapsulate both hydrophilic and lipophilic drugs. However, there is possibility of coalescence of the nanodroplets during the evaporation process.

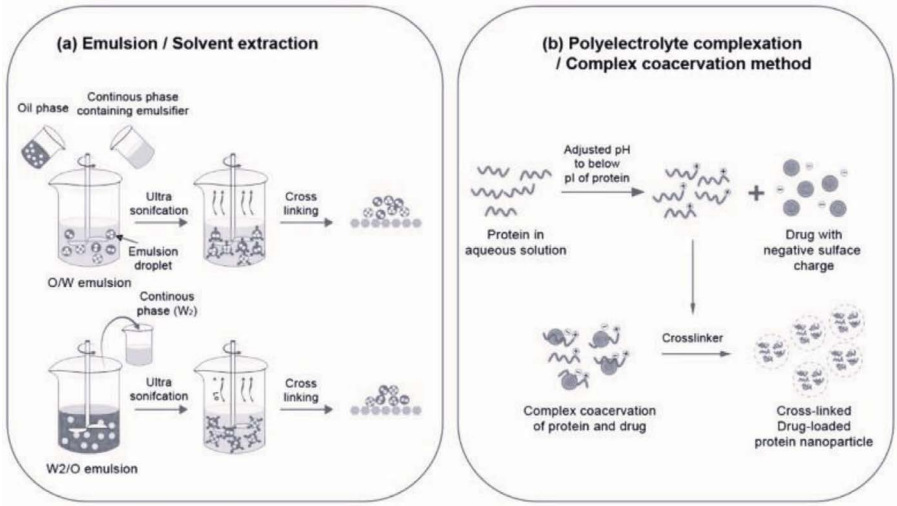



**FIGURE 8.2** (a) The emulsion process is a method of forming nanoparticles by removing solvent/non-solvent after an emulsion system is formed by dispersing via mechanical agitation or ultrasonic waves. Moreover, a double emulsion method was also used. (b) The complex coacervation method adjusts the pH to produce the protein cationic or anionic and then interacts with other polymers to produce nanoparticles. (From https://pmc.ncbi.nlm.nih.gov/articles/PMC7407889/figure/pharmaceutics-12-00604-f003/ accessed on 8 Nov. 2023.)

#### 8.4.1.2 Double Emulsification Technique

Double emulsion method was used to provide high EE of the payload (Wang and Uludag, 2008). In the W/O/W double emulsion method, the primary W/O emulsion is dispersed in a second water phase using a surfactant for stabilizing the emulsion. Thereafter, an emulsion system is formed, and organic solvent O is removed to preserve the nanoparticles in an aqueous buffer (Figure 8.2a). Synthesis of double emulsions of nanoparticles is a fast and cost-effective method. Emulsification is usually thermodynamically unstable and particle bonding occurs to minimize the free energy of the system (Müller et al., 1996). Therefore, a surfactant and a stabilizer are needed to stabilize the emulsion particles. Surfactants are likely to affect drug-matrix interactions and the rate of drug release in a physiological environment. For protein nanoparticles, protein concentration and relative volume ratio of water and oil phases are important parameters. Common method where w/o emulsion is formed between aqueous drug phase with PLGA polymer organic solvent phase, which is dispersed in aqueous continuous phase polymer solution to form double multiple emulsion w/o/w, from where organic solvent phase is removed to form nanoparticles (Zhu et al., 2005; Jeong et al., 2008) (Figure 8.2a).

#### 8.4.1.3 Emulsion Coacervation

Phase separation is broadly applied to various processes, such as formation of solid or liquid phase from a solution. Examples are crystallization of a salt or precipitation of a polymer due to removal of some solvent from solution. The new phase is

physically distinct having different properties compared with solution (Figure 8.2b). It is separated by mechanical means. The word coacervate was first noted in 1623. This word may be defined in the present context as "an aggregate of colloidal droplets" (as of two hydrophilic sols or of a sol and ions of opposite charge) held together by electrostatic attractive forces (Nairn, 1995). Coacervation is a term that is used to describe the formation of a coacervate related to phase separation. It is applied to the separation of a colloid from solution into a phase rich in the colloid called the coacervate and the remaining phase which is poor in colloid (Bungenberg de Jong, 1949a). The coacervate is different from original solution as it forms a separate liquid layer, but with stirring may form droplets suspended in the polymer-poor phase. Furthermore, it is usually more viscous and more concentrated. It often has the property of binding or adsorbing onto, or engulfing a solid or liquid which may be added in to the system.

Coacervation has also been defined as:

- the partial miscibility of two or more optically isotropic liquids, where one is in the colloidal state.
- production by coagulation of a hydrophilic sol of a liquid phase, which often appears as viscous drops, not as a continuous liquid phase (Considine, 1983). Luzzi (1976) indicated that a solid precipitate is not formed, but forms a polymer phase consisting of liquid droplets.

Bungenberg de Jong (1949a) indicated that simple coacervation is concerned with nonionized groups on the macromolecule and complex coacervation is concerned with the charges on the macromolecule and the formation of salt bonds. Luzzi (1976) and Deasy (1984) indicate that simple coacervation usually deals with systems containing only one colloidal solute and complex coacervation involves systems containing more than one colloid, the solvent being water.

Arshady (1990a) uses the term coacervation to include the use of both water and non-aqueous systems and describes simple coacervation and complex coacervation in terms of one or two polymers in aqueous systems, respectively. Another definition of a coacervate as proposed by Ecanow et al. (1990) is that phase most dissimilar to water in its physical chemical properties. Deasy (1984) has pointed out that the solvent is continuous on both sides of the interface and that the polymer or macromolecule is able to diffuse between the two phases. Some authors separate the discussion of microencapsulation by coacervation, which includes the use of temperature change or incompatible solvent addition, from other phase separation methods such as the use of emulsification and/or solvent removal to prepare a polymer-rich phase (Arshady, 1990a, 1990b; Watts et al., 1990). Other authors include all these processes in the term coacervation-phase separation to indicate that a new phase, a polymer-rich phase, is being formed. It is also a process applied to a polymer solution, usually dilute, to reduce solubility in the system to such a degree that appropriate phase separation of the polymer takes place and there is formation of a polymer-rich phase. This polymer-rich phase may be used to treat pharmaceuticals and other substances. Alternatively, the formation of the colloid-rich phase may result in flocculation and the colloid is

present in a higher dispersed state and is usually not satisfactory for encapsulation (Bungenberg de Jong, 1949a).

Different processes involved are:

1. A single wall-forming polymer soluble in water addition of a miscible liquid, a non-solvent for the polymer, addition of a water-soluble salt, change of temperature, addition of an incompatible or non-wall-forming polymer, adjustment of pH and addition of reacting ions.
2. Two or more wall-forming polymers soluble in water: pH adjustment, dilution and/or temperature change, addition of an incompatible or non-wall-forming polymer, three wall-forming polymers.
3. A single wall-forming polymer soluble in an organic liquid addition of a miscible liquid, a non-solvent for the polymer, change of temperature, addition of an incompatible or non-wall-forming polymer, evaporation with a miscible liquid, a non-solvent for the polymer, evaporation with an immiscible polar liquid, a non-solvent for the polymer, evaporation or removal with an immiscible organic liquid, a non-solvent for the polymer.
4. Two wall-forming polymers soluble in organic liquids.
5. One wall-forming polymer soluble in water and one soluble in an organic liquid.

Coacervation is a method of precipitating nanoscale or micron-scale biodegradable polymers by liquid-liquid phase separation techniques. It can facilitate polymer interactions by changing the ionic strength within the system, changing the temperature or adding a non-solvent to precipitate the polymer (https://www.mdpi.com/1422-0067/24/5/4333#:~:text=Coacervation%20is%20a%20method%20for,precipitate%20the%20polymer%20%5B61%5D; Wang et al., 2022). Coacervation methods can be mono-coacervation and complex-coacervation methods. In mono-coacervation method, hydrophilic electrolyte or a non-electrolyte coagulant is added to reduce solubility of nanomaterials, which cause them to agglomerate into microparticles. The complex-coacervation method uses two polymer materials having opposite charges to form vesicle, which reduces the solubility and causes the microparticles to agglomerate and precipitate out of the solvent (Makadia and Siegel, 2011; Butreddy et al., 2021).

When using single and double emulsion method of particle preparation, the choice of solvent and mixing rate can affect the EE and final particle size. So the particles are often used as injectable microsphere formulations (Makadia and Siegel, 2011). Furthermore, the coacervation method is a process focused on the preparation of micron-scale biodegradable polymer encapsulated formulations through liquid-liquid phase separation techniques. The desired morphology and size of the microspheres can be obtained by adjusting processing parameters such as polymer concentration, quenching temperature, quenching time, and solvent composition (Thomasin et al., 1998; Graham et al., 1999). Different synthetic methods allow easy processing and fabrication of PLGA particles. This can be adapted to deliver different drugs as well as different delivery methods. This reinforces the fact that PLGA polymer is a very promising drug delivery vehicle.

#### 8.4.1.4 Emulsification – Reverse Salting-Out

The salting-out method is based on the separation of water-miscible solvent from aqueous solution, through salting-out effect that results in the formations of nanospheres (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464532/ accessed on 10 Nov. 2023) (Wang et al., 2016). This is formulated from a water-miscible polymer solvent, such as acetone or ethanol. The aqueous phase contains a gel, the salting-out agent and a colloidal stabilizer (Lim and Hamid, 2018). Examples of suitable salting-out agents include electrolytes, such as magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), or magnesium acetate [$Mg(CH_3COO)_2$], as well as non-electrolytes, for example, sucrose (Pal et al., 2011). The miscibility of acetone and water is reduced by saturating the aqueous phase, which allows the formation of an o/w emulsion from the other miscible phases (Vauthier and Bouchemal, 2009). The o/w emulsion is prepared with intense stirring at room temperature. Thereafter, the emulsion is diluted using an appropriate volume of deionized water, or by aqueous solution to allow the diffusion of the organic solvent to the external phase. It results in precipitation of the polymer leading to formation of nanospheres. The remaining solvent and salting-out agent are eliminated by crossflow dialysis or diafiltration. The condition of complete miscibility between the organic solvent and water is not essential, but it simplifies the execution process (Krishnamoorthy and Mahalingam, 2015). This method is schematically presented in Figure 8.3. The nanospheres are obtained in the range of 170–900 nm. By varying polymer concentration of the internal phase/volume of the external phase, the average particle size can be adjusted between 200 and 500 nm (Crucho and Barros, 2017).

Minimization of the stress to fragile drugs, high loading efficiency, and easy to scale-up. However, there are possibilities of incompatibility between the salts and the drugs and purification is needed to remove electrolytes.

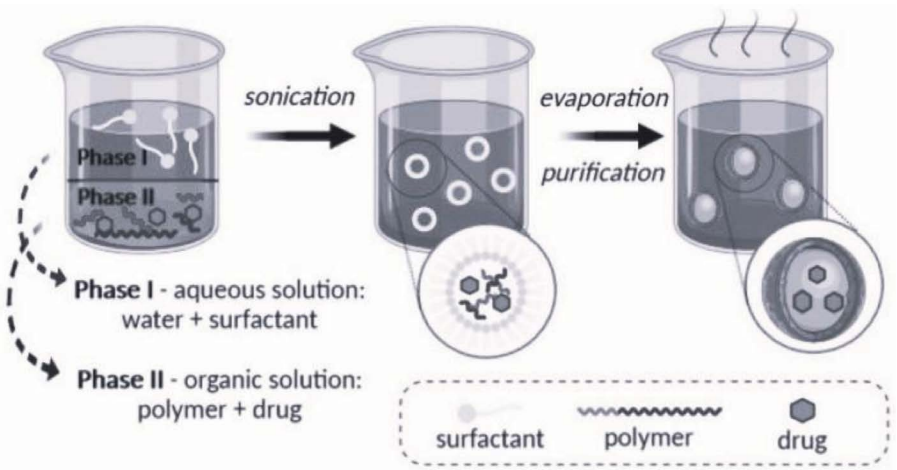



**FIGURE 8.3** Schematic representation of the emulsification/reverse salting-out method. (From https://pmc.ncbi.nlm.nih.gov/articles/PMC7464532/figure/molecules-25-03731-f002/ accessed on 11 Nov. 2023.)

#### 8.4.1.5 Poly-ionic Complex and Ionic Gelation or Nanogels

Charged polymers can be used to form nanoparticles. Polyelectrolytes are one such example: these polymers have a repeating electrolyte group, giving them charge that varies with pH. Polyelectrolytes have been incorporated in numerous NP formulations to improve properties such as bioavailability (Cao et al., 2019) and mucosal transport (Tian et al., 2018). They are also inherently responsive and can be useful for intracellular delivery.

Polyelectrolyte complexes are formed between polyamines and nucleic acids due to complementary charge annealing (Howard and Kjems, 2007; de Martimprey et al., 2009). These nanospheres are also named as nanoplexes. These are widely developed as drug carriers. These make possible *in vivo* delivery of nucleic acids like plasmid genes, siRNA and antisense oligodeoxynucleotides. Here, the drug being the nucleic acid is incorporated in the nanocarrier as a part of the drug delivery device. The main formulation parameter is to take into account to obtain the nanospheres within the desired size range is given by the N/P ratio. N/P is defined as the ratio of the number of amine groups of the polycation divided by the number of phosphate groups of the nucleic acid. So, the "N" value represents the number of positive charges involved in the formation of the complex, while the "P" value represents the number of negative charges. In general, the net charge of the polyplexes appears positive when the N/P ratio is above 1.

The internal structure of polyplexes are gel structures. In this structure, the complexed polyelectrolyte chains are swollen by water molecules. Polycations that are used to prepare polyplexes are poly(ethylenimine) (PEI), poly (lysine) (PLL), poly(ornithine), or chitosan. One type of polyamines includes dendrimers that form very small complex with nucleic acids. Nanosized polyelectrolyte complexes were formed with other types of polyelectrolytes. For instance, Rajaonarivony et al. (1993) have pointed out the formation of aggregates in the nanosized ranged after the mixing alginate, a negatively charged polysaccharide, with polylysine, a positively charged peptide, in very well-defined concentrations of the two polyelectrolytes.

A more extensive work has considered obtaining nanoparticles by polyelectrolyte complex formation from polysaccharides of complementary charged (Schatz et al., 2004; Drogoz et al., 2007). The polysaccharides were dextran sulfate containing negatively charged sulfate groups and chitosan containing positively charged amino groups. In this case, the nanoparticles are elaborated by a one shot addition of the polyelectrolyte used in default in the one used in excess. According to the nature of the polyelectrolyte used in excess, either positively or negatively charged nanoparticles can be synthesized. This is an interesting property because it can affect the way the nanoparticles will interact with cells.

The nanoparticles featured a core/shell structure. The hydrophobic core is composed by the complexed segments whereas the excess of component not incorporated in the polyelectrolyte complex is segregated in the outer shell ensuring the colloidal stabilization of the nanoparticles against coagulation and conferring the charge of the nanoparticle surface. The molecular weight of the two polyelectrolytes influences the size of the nanoparticles. A correlation between the chain length ratio of the polyelectrolytes and the size of the nanoparticle formed could be established on the basis of a host/guest concept. According to this concept, the polyelectrolyte of the highest molecular weight serves as host for the guest polyelectrolyte of lower molecular

weight (Schatz et al., 2004). The mechanism of formation of the nanoparticles was found to vary according to the polyelectrolyte used in excess during the preparation. This was attributed to the differences in chemical reactivity of the ions of the polycation in excess and to the flexibility of the macromolecular chains.

Polymers play a major role for designing an oral delivery system, use of natural polymers like chitosan and alginates instead of toxic chemicals polymers in oral delivery system enhances the permeation effect, enzyme inhibitory ability and mucoadhesive property of the drug (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4352224/ accessed on 11 Nov. 2023). Briefly, based on the solubility of the drug and polymer, they are dissolved in weak acidic medium or water and the resultant solution is added to the solution containing counter ions and stabilizer drop wise under constant stirring. Spherical shaped particles are formed due to the complexation of oppositely charged species which results in gelation and precipitation. The particle size is reduced to the nanometric range buy sonicating the resultant solution. The nanosuspension produced is freeze dried using 5% mannitol as a cryoprotectant to obtain a fine powder of Nanoparticles (Fan et al., 2012; Rajan and Raj, 2012).

There is a possibility to use natural macromolecules, which are hydrophilic and biocompatible. However, it is limited to the encapsulation of hydrophilic drugs.

- One step procedures method based on ionic gelation: Organic solvent free method and there is possibility to control the release of a drug encapsulated in the nanoparticles upon the action of a pH or an ion concentration variation stimulus. Possible particle disintegration due to the weakness of the ionic interactions.
- Formation of nanoparticles from neutral nanogels: Organic solvent free method for controlled release of the drug. However, it is not yet applicable to hydrophilic drugs.

### 8.4.2 Solvent Injection Method

Water-miscible organic solvent (like ethanol) containing water-insoluble active & carrier (lipid/polymer) is injected in aqueous phase, under high shear, resulting into precipitation in form of nanosuspension/polymer nanoparticle/liposome/solid LNP. It is also called as Nanoprecipitation of a polymer (Zili et al. 2005; Limayem et al., 2006): This process has high simplicity, it is fast and reproducible, with low polymer concentration in the organic phase and low concentrations of surfactants used. Easy to scale-up, liquid antisolvent precipitation method is the most effective method to produce drug nanoparticles. Drugs are dissolved in a solvent and mixed with an antisolvent (e.g., water), which results in increased supersaturation. This leads to the formation of a large number of small particles. Compared to other methods of nanosizing, this is an easy, cost-effective, and scalable process for manufacturing.

### 8.4.3 "In Situ" Polymerization and Interfacial Polycondensation

To produce nanoparticles by in situ polymerization, a monomer is added in the emulsion instead of a polymer solution and the polymer forms by polymerization to give

birth of nanoparticles. While very well-characterized nanospheres can be produced by polymerization of various types of monomers in emulsions or miniemulsions, nanocapsules are obtained by performing interfacial polymerization or polycondensation reactions in emulsions or microemulsions (Gasco and Trotta, 1986). So far, only a few monomers were found suitable to produce nanospheres and nanocapsules for *in vivo* applications as drug delivery carriers. An abundant literature is devoted to nanoparticles made from alkylcyanoacrylates (Vauthier et al., 2007).

All kinds of nanoparticles, that is, nanospheres, oil-containing nanocapsules, and water-containing nanocapsules, can be prepared by in situ polymerization of such monomers. This is an advantage because a wide range of drugs can be associated with poly(alkylcyanoacrylate) nanoparticles. This actually includes small and large drug molecules which are either hydrophilic or lipophilic. The different methods of polymerization developed to prepare poly(alkylcyanoacrylate) nanoparticles are now quite well understood leading to the obtaining of nanoparticles with very defined properties. The majority of poly(alkylcyanoacrylate) nanospheres and nanocapsules are obtained by the anionic polymerization of the corresponding monomer which is spontaneously initiated by hydroxyl groups of water or by any types of nucleophilic groups found on molecules dissolved in the polymerization system. Easy method of obtaining core-shell tuned nanoparticles is by controlling the size of the nanoparticles by using surfactant. (Vauthier and Bouchemal, 2009). Low concentrations of surfactants are required and are limited to the encapsulation of lipophilic drugs. Modulation of the nanocapsule thickness is achieved by varying the monomer concentration and purification is needed.

### 8.4.4 Self-Assembling Polyelectrolyte Complex and Complex Coacervation

#### 8.4.4.1 Self-Assembled Micellar Systems

PMs are other nanostructures, which have gained attention for encapsulating and delivering hydrophobic drugs (Figure 8.4a). PMs, with diameters ranging from 10 to 100 nm, are self-assembled nanostructures formed in aqueous solutions characterized by a core-shell structure; the inner core is composed of the hydrophobic regions which encapsulate the poorly water-soluble drug, whereas the outer shell or corona of the hydrophilic block of the copolymer protects the drug from the aqueous environment (Xu et al., 2013). PMs are drug delivery system used to improve solubility of poorly water-soluble drugs. These have advantage over normal micelles, as they solve the problem of precipitation of drug when the micelles get diluted in the human circulatory system, thereby reducing the chances of embolism. This is due to the fact that PMs have very low critical micellar concentration.

PMs always contain a nonionic water-soluble compound and an ionic compound that can be neutralized by an oppositely charged surfactant to form a hydrophobic core. The electrostatic interaction between the ionic segment of the block polymer and the surfactant group changes these segments from water-soluble to water-insoluble, leading to a hydrophobic core in the micelles (Ahmad et al., 2010). Self-assembled PMs have special characteristics as delivery systems, such as high loading capacity and improved solubility of drugs, decreased systemic unfavorable effects, enhanced permeability and retention effect, and their possible modification of physicochemical characteristics (Vonarbourg et al., 2006).

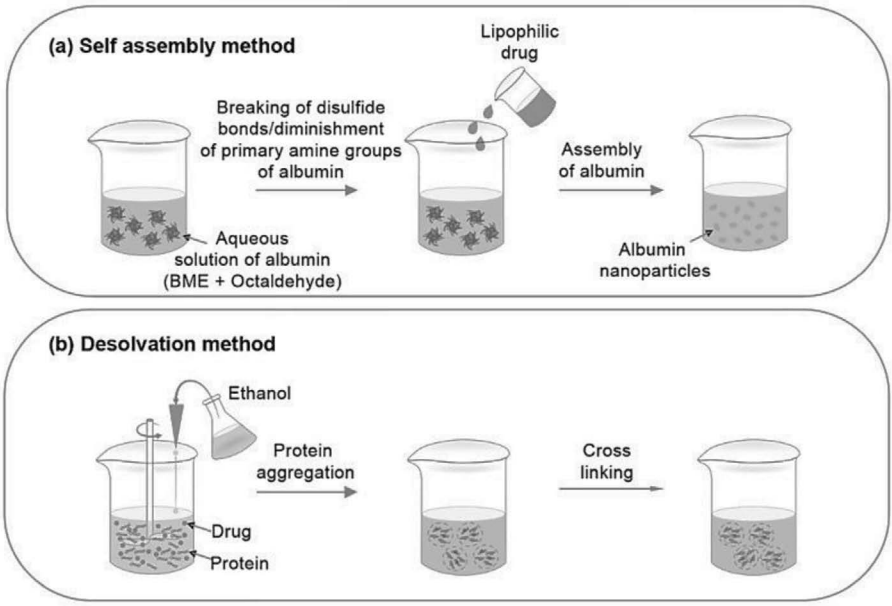



**FIGURE 8.4** (a) Self-assembly method: Individual protein chains are dissolved in an aqueous solution, where CMT exceeds CMC to spontaneously generate protein micelles which are nanosized aggregates. (b) Desolvation method: Nanoparticles are prepared by adding a desolvating agent to a protein solution containing drugs. (From https://pmc.ncbi.nlm.nih.gov/articles/PMC7407889/figure/pharmaceutics-12-00604-f005/ accessed on 08 Nov. 2023.)

The PMs carriers for the purpose of drug delivery are synthesized under three distinct designs:

- The micelle-forming polymer–drug conjugates, in which the drug incorporation within the micellar carrier is achieved through the formation of hydrolysable chemical bonds between the functional groups of the polymeric backbone and the drug (Girase et al., 2020);
- The polymeric micellar nano-containers design, which can be achieved only if the block copolymer and the drug are water soluble. In this system, the formation of hydrophobic interactions or hydrogen bonds between the micelle-forming block copolymer and drug provides the basis for the solubilization of drugs in the PMs (Shahin and Lavasanifar, 2010); and,
- Finally, the polyion complex micelles, in which the incorporation and delivery of different therapeutic moieties that carry charge are needed to promote the electrostatic interactions between oppositely charged polymer and drug combination (Chen et al., 2018).

The hydrophobic core of the micelles is used to encapsulate poorly soluble drugs, thereby decreasing their elimination from the body, while the polar hydrophilic exterior surface allows dissolution in an aqueous medium (Hammad and Muller, 1998).

The core-shell structure of the micelles mimics the naturally occurring transport system in the body, also facilitating the absorption and distribution of entrapped drugs (Kwon and Okano, 1996; Kabanov and Alakhov, 2002). Estradiol (Estrasorb™) is a FDA-approved micellar formulation in the market. It is used in the treatment of moderate to severe vasomotor symptoms of menopause (Bobo et al., 2016). Many micellar formulations are in clinical trials. For example, BIND-104 is used in prostate cancer therapeutics. It is a Docetaxel-encapsulated micellar formulation made up of degradable and hydrophobic polymeric core and hydrophilic PEG shell (Hrkach et al., 2012). Other examples of micellar formulations currently in clinical trials are CriPec® (Rijcken et al., 2005) and CALAA-01 (Davis, 2009).

Protein micelle, a type of PM can be spontaneously produced when individual protein chains are dissolved in a solution exceeding the critical micelle concentration (CMC) and at the critical solution temperature (CST) to form nanosized aggregates (Batrakova et al., 2006). By forming a bridge between the chains, micelles can be stabilized via the solidification process. Albumin, a hydrophilic protein, can be given an amphiphilic property through the hydrophobic modification process. Hydrophobically modified proteins can be self-assembled into micelle nanoparticles upon its addition to aqueous solutions. Moreover, hydrophobic cores can serve as a conduit for active molecules.

#### 8.4.4.2 Complex Coacervation and Desolvation Techniques

It is easy to achieve, but there is necessity to optimize the ratio between negatively and positively charged molecules. According to the nature of the polyelectrolyte used in excess, either positively or negatively charged nanoparticles can be synthesized (Vauthier and Bouchemal, 2009). Since proteins are amphoteric with multiple charged functional groups, they can be made cationic or anionic by adjusting various factors such as the pH of the protein. The charged protein can interact electrostatically with other polymeric electrolytes. The pH-dependent electrostatic interaction between proteins and other polymers can be used to design stable biocompatible nanoparticles and coacervates to facilitate the controlled transmission of bioactive treatments and DNA (Truong-Le et al., 1998). A cationic protein polymer is commonly used to form a complex with anion oxygen atoms in a phospholipid backbone of oligonucleotides to reduce DNA/RNA therapeutic molecules shaped like long strings to nanoparticles (Nisha et al., 2004) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7407889/ accessed on 08 Nov. 2023).

PMs and desolvation of polymeric systems can lead to formation of nanoparticles. Protein micelle can be spontaneously produced when individual protein chains are dissolved in a solution exceeding the CMC and at the CST to form nanosized aggregates (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7407889/ accessed on 08 Nov. 2023) (Figure 8.4b). By forming a bridge between the chains, micelles can be stabilized via the solidification process. Albumin, a hydrophilic protein, can be given an amphiphilic property through the hydrophobic modification process. Hydrophobically modified proteins can be self-assembled into micelle nanoparticles upon its addition to aqueous solutions. Moreover, hydrophobic cores can serve as a conduit for active molecules. Gong et al. synthesized protein micelle nanoparticles based on the specific reaction between the primary amino group of albumin and octaldehyde and they

were able to form a core-shell nanomicelle (Gong et al., 2009). It was dissolved in water, integrated, and filtered through a 0.45-μm pore-sized fine porous membrane. The paclitaxel was successfully loaded into the protein micelle through the dialysis method with about 33.1% high drug loading and 90.5% high EE. Sabra et al. (2018) manufactured a zein-lactoferrin (zein-Lf) micelle that highly encapsulated hydrophobic drugs, such as rapamycin (RAP) and wogin (WOG), through the hydrophobic coupling reaction. Hydrophilic Lf was included to expand the spectrum of tumor targets. GA was used as a crosslinking agent. Zein-Lf micelle displayed a slow spread of the RAP from the zein core with relatively fast WOG release. This sequential release may lead to efficiency in pump infusion by WOG, thereby sensing tumor cells to RA action. Zein-Lf micelle also showed improved stability and excellent hemocompatibility. Hence, the combined nano-delivery system maximized the synthetic cytotoxicity of RAP and WOG in MCF-7 breast cancer cells.

Desolvation is the most commonly used method in terms of the production of protein-based nanoparticles (Weber et al., 2000; Zhao et al., 2015). The desolvation method allows for the synthesis of nanoparticles through a simple process of adding desolvating agents, such as ethanol and acetone, to protein solutions containing drugs. Desolvating agents change the protein structure and reduce the solubility of the protein, thereby leading to the formation of precipitation in the form of protein nanoparticles (Figure 8.4). The formation of particles during which the particle size increases up to a certain level and is achieved by a gradual increase in the number of the same size of particles (Zhao et al., 2015). Once nanoparticles are formed, they are bridged by bridging agents, such as GA. Protein nanoparticles manufactured by the desolvation method can adjust particle size according to conditions (Langer et al., 2008). Protein concentration, desolating agent additive speed, pH, and temperature are the main factors affecting particle size. In particular, high pH and low-protein concentration can produce smaller nanoparticles. The desolvation method is not directly involved in the synthesis of nanoparticles, but it is a self-assembly method because the protein is concentrated by reducing the solubility of the protein using the desolvating agent (Figure 8.4b). The method is widely used in the generation of nanoparticles using albumin. Particle size was found to be dependent on the protein concentration, volume, pH, and temperature of the desolvating agents for the volume of protein solution. The nanoparticles were stabilized by the bridging with glutaraldehyde. The nanoparticle diameter was within the range of 230–310 nm, depending on the wt% of 30Kc19-HSA, while the loading efficiency was within the range of 80% to 95%.

### 8.4.5 Polymer Coating (Calvo et al., 1997) and Layer-by-Layer Method (Chen et al., 2009; Agarwal et al., 2008)

The layer-by-layer (LbL) deposition procedure is a versatile technique, based on the sequential deposition of complementary molecular species on colloidal surfaces. The attachment of the LbL coating upon drug nanocore surfaces creates a multilayered thin core-shell nanocapsule. Owing to the electrostatic main driving force for multilayer LbL shell build-up, a wide variety of shell constituents can be used, ranging from synthetic to naturally derived polyelectrolytes, lipids, enzymes, viruses,

colloids, and other active compounds. These multilayered films allow the combination of several different materials at the nanoscale in a single unit, giving rise to novel and multifunctional architectures capable of performing numerous tasks required for systemic drug delivery. These features allow the possibility of drug protection and high drug loadings, together with drug release tuning capacities from the core by modulating the LbL coating permeability (Santos et al., 2018).

Among the several charged materials introduced for LbL assembly onto the surface of drug nanocores, polyelectrolytes have been the most frequently applied. In all cases, coating materials can be categorized according to their origin, namely synthetic (non- and biodegradable) or natural. A range of different polyelectrolytes have been used. Standard synthetic polyelectrolytes include poly(styrene sulfonate) (PSS), poly(dimethyldiallylammonium chloride) (PDDA), poly(ethylenimine) (PEI), poly(N-isopropyl acrylamide) (PNIPAM), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMA), poly(vinyl sulfate) (PVS), and poly(allylamine) (PAH) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4464506/ accessed on 24 Dec. 2023). Natural polyelectrolytes include polysaccharides, including commonly alginic acid, chitosan, cellulose sulfate, dextran sulfate, heparin, protamine sulfate (PS), and carboxymethylcellulose (de Villiers et al., 2011; Ariga et al., 2011). Some of the cation-anion pair includes:

The choice of coating materials for LbL shell assembly is confined to low-molecular-weight polyelectrolytes (65 kDa), due to the restricted space for polymer chain adsorption available for nanoparticles. This behaves in contrast to the case of microparticles, that are larger, exhibiting approximate characteristics to a flat surface (Shutava et al., 2012). The peculiarity of the LbL assembly process on small nanoparticles pertains to the confined space available for polyelectrolyte chain adsorption on the surface of nanoparticles, as well as the extreme influence of the ionic strength of the solution. As a consequence, the tails of polyelectrolytes protrude into solution (Chodanowski and Stoll, 2001), promoting aggregation. Recently, a work of our research group revealed that low-molecular-weight polymers promoted a decrease in aggregation. In fact, the use of a PSS endowed with a higher molecular weight led to enhanced aggregation, which was reversed in the following layer by using lower-molecular-weight PAH. This accordingly created a particle size oscillation during LbL assembly, depending on the polyelectrolyte nature of the outer layer of the LbL shell (Santos et al., 2015).

A very interesting fact of LbL assembly upon nanocores is the possibility and, in many cases, the demand for charged polyelectrolytes adsorption from solutions containing an overload of uncharged, occasionally polymeric, pharmaceutical excipients used for preparation and/or stabilization of nanoparticles, such as polyvinylpyrrolidone (PVP), glycerol, polyethylene glycol (PEG), and sucrose. The presence of water-soluble polymers when performing LbL multilayers adsorption onto the surface on nanoparticles can thereby markedly contribute to modification and stabilization of the formulations (Shutava et al., 2012).

LbL modification is an effective way to tune the properties of particles. However, the traditional LbL process involves repeated washing steps which are not compatible with nanoparticles with a partial solubility. Elbaz et al. (2023) demonstrated the use of a titration method for producing LbL coatings onto a nanosuspension

of curcumin (a compound with a limited aqueous solubility). LbL can be used to enhance the release behavior and stability of the curcumin in a nanosuspension form. Coated nanosuspension samples were produced using biocompatible and biodegradable polyelectrolytes, poly-L-arginine, and sodium alginate and were sizes approximately 500 nm. A stimuli-responsive nanosuspension was prepared by coating the nanosuspension with five layers of poly-L-arginine and alginate and Eudragit L100 as an outmost layer. The *in vitro* release of these nanosuspensions revealed that the use of a pH-responsive layer (Eudragit L100) as an outermost shell resulted in delay the release of curcumin (5%) in acidic pH and facilitates its release in neutral pH (11%) over 72 hours. Additionally, the bioaccessibility study showed that increasing the number of layers resulted in increasing the stability curcumin in nanosuspension when exposed to conditions that mimic the gastrointestinal tract from 20% for uncoated nanosuspension to 40% and 90% for 4-layered coated nanosuspension and stimuli-responsive (6-layered) coated nanosuspension, respectively. The cytotoxicity of LbL-coating of nanosuspension revealed a reduction in the toxicity of curcumin. This work shows how a titrated LbL modification approach could be used to tailor the stability and the release behavior of nanosuspensions for oral drug delivery applications.

LbL method is a thin-film fabrication technique. The films are formed by depositing alternating layers of oppositely charged materials. The layers can be performed in different ways, for example, dip coating, spin-coating, spray-coating. Various materials can be deposited by LbL method including polyions, metals, ceramics, nanoparticles, and biological molecules (https://chobotix.cz/research-2/finished-or-inactive-projects/layer-by-layer-method/ accessed on 22 Nov. 2023).

### 8.4.6 Dialysis Technique

Dialysis technique is a simple and effective technique for the preparation of polymeric nanoparticles with narrow distribution (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4352224/ accessed on 11 Nov. 2023). In this technique, drug and polymers are placed inside a dialysis tube/membrane (with appropriate molecular weight cut off), after dissolving in water-miscible organic solvents. The organic phase diffuses out through the dialysis tube/membrane into the aqueous phase, due to decrease in the interfacial tension between them. After the displacement of solvent inside the membrane, there is progressive aggregation of polymer due to the loss of solubility, which leads to formation of homogeneous suspension of nanoparticles. The nanosuspension produced is freeze dried using 5% mannitol as a cryoprotectant to obtain a fine powder of nanoparticles (Liu et al., 2007; Choi and Kim, 2007; Rao and Geckeler, 2011).

## 8.5 PRODUCTION OF POLYMERIC NANOPARTICLES: GENERAL CONSIDERATIONS

The placement of various components of a formulation together through a step-by-step manner is the traditional pharmaceutical batch processing. As materials move from one step to the next, the batch in production must terminate before proceeding

to the following one (https://www.generalkinematics.com/blog/batch-vs-continuous-pharmaceutical-manufacturing/ accessed on 24 Dec. 2023). In contrast, the continuous-flow processes involve moving one work unit at a time between each process step without interruption in time, sequence, substance, or extension. The main advantage of batch production is that the initial setup costs are less expensive. Also, uniquely batches can be easily customized. Specific quantities of certain product can be adjusted based on changing market demands (kinematics) in batch production. A complete and thorough cleaning of the equipment after each produced batch is required to be carried out, which takes additional time. Further, it also requires challenging documentation and validation methods that affects production costs (FDA Guidance for Industry, Process Validation: General Principles and Practices, January 2011, Current Good Manufacturing Practices (CGMP), Revision 1).

For large-scale of nanoparticle's production, API loading achieved is an important parameter. Only manufacturing technology alone is not sufficient to decide the EE. There are many formulation and process parameters, which are equally influential for efficient API loading. The process should not alter the EE as well as other properties of PLGA nanoparticles upon scaling-up for large-scale production.

### 8.5.1 Mixing Considerations

#### 8.5.1.1 Conventional Methods: Simple Mechanical Stirring and Probe Sonication

These are straightforward and broadly exploited techniques due to their versatility and ease of operation (Schiller et al., 2015; Huang and Zhang, 2018; Operti et al., 2018; Hernandez-Giottonini et al., 2020). Nanoprecipitation, salting-out, and dialysis using mechanical stirring are some conventional methods by which nanoparticles are produced. Emulsion solvent evaporation/diffusion operating probe sonication process is also used for nanoparticle production. These said technologies are used only for small batch preparations. Scaling up of such production process can alter the formulation characteristics, which is a main drawback. In probe sonication, additionally direct contact of the immersed probe with the sample can lead to contamination like by heavy metals. This problem can be avoided by indirect sonication strategies when the energy is distributed to the sample through the sample container itself (Freitas et al., 2006; Schiller et al., 2015). Sensitive APIs are also affected by harsh homogenization conditions and high temperature (Schiller et al., 2015).

#### 8.5.1.2 Alternate High-Impact Methods

The three techniques commonly used for the production of micron-sized particles are HSM, HPH, and microfluidization. There is possibility to reach nanosized scale by adjusting the process and formulation parameters (Dong et al., 2007; Sani et al., 2009; Operti et al., 2018; Tukulula et al., 2018). These are simple and easy-to-reproduce processes. This gives a broad range of achievable particle sizes and the possibility of inline operation.

During scale-up, high temperature is created in the devices. Also, longer process times plus harsh homogenization forces affect sensitive materials. Moreover, proper cleaning of the equipment and cleaning validation, is also a challenge. This

is aggravated due to the complex geometry of the rotor blades of the shear mixers. The tiny channels present in the reaction chambers of microfluidizers and pressure homogenizers also create problem for cleaning. Also, high shear speed might create disturbance in flow rates (e.g., sucking effects). Microfluidizers can suffer from potential blockage of the small channels due to precipitation of PLGA particles or due to too viscous obtained suspension, when connecting the system inline to other equipment homogenizers (Operti et al., 2018).

HSM, sonication (direct or indirect), HPH, microfluidization, microfluidics, and millifluidics which are primarily designed for batch processing, can also be adapted for inline operation, which makes them more attractive from an industrial standpoint. And the manufacturing can be implemented in either mode of operation (i.e., batch or continuous). Like, paclitaxel protein-bound particles (nab paclitaxel; ABI-007; Abraxane) is a novel formulation of paclitaxel that does not employ the CrEL solvent system. The nanoparticle colloidal suspension of paclitaxel can be produced by high-pressure homogenization (HPH) in the presence of HSA at a concentration of 3% to 4% (similar to that of albumin concentration in the blood) (Ibrahim et al., 2002).

Nanoparticle albumin-bound (nab) drug delivery, using human protein albumin drug delivery reduces side effects but it can be difficult to create. Solvent-based paclitaxel causes side effects for patients. Achieving small and uniform particle size is a key step in making nanoparticle albumin-bound (nab) drug delivery systems. Creating the right manufacturing process that enables downstream processes to be more effective and ensuring the final product meets target physiochemical properties can be difficult to achieve if the wrong technology is applied. Microfluidizer® technology delivers precisely controlled high shear forces creating nanoparticles at the target size and distribution. The processors produce best-in-class stable nanoemulsions offering an effective drug delivery system for nab paclitaxel. The process is linearly scalable from the lab to production. Formulating nab paclitaxel as a nanoemulsion is a superior methodology that ensures the paclitaxel and albumin are combined correctly. The active ingredient, which is the paclitaxel, is dissolved in an organic solvent to form an oil phase. This is then mixed with an aqueous phase – which is the albumin solution. This produces a coarse emulsion which is then processed using high shear to create a nanoemulsion. This high shear is delivered via Microfluidizer® technology, which produces the target droplet size with a mean particle size of 130 nm and a tight distribution. The resulting nanoemulsion undergoes further processing to remove any traces of the organic solvent, it is then submitted to sterilization and lyophilization steps to create the final product in the form of powder (https://www.microfluidics-mpt.com/applications/nanoparticle-albumin-bound-nab-drug-delivery accessed on 10 Nov. 2023).

Alternatively, Abraxane (ABI-007) is produced by patented nanoparticle albumin-bound (nab) technology, which is based on an emulsion-evaporation crosslinking method as shown in Figure 8.5 (Fu et al., 2009). The oil phase is added drop by drop to the aqueous phase, HSA solution (1% w/v) presaturated with 1% chloroform. A crude emulsion is formed when the mixture subjected to a low shear forces at low rotating speed. A final emulsion is produced by homogenizing the crude emulsion at a high pressure and recycling the emulsion through homogenizer. The albumin

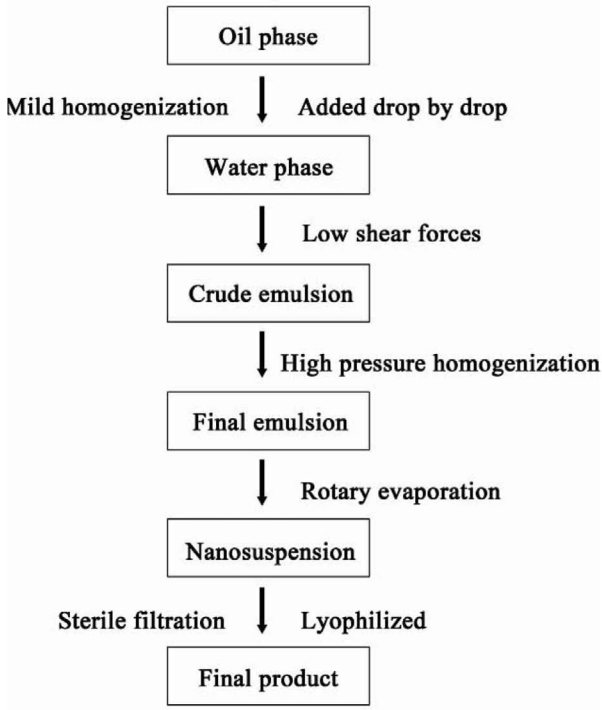



**FIGURE 8.5** Schematic of preparation processes of ABI-007. (The oil phase employed generally contains 30-mg paclitaxel dissolved in 0.55-mL chloroform, another 0.05-mL ethanol is also added in the oil phase. The water phase is 29.4-mL human serum albumin (HSA) solution (1% w/v) presaturated with 1% chloroform.)

nanosuspensions, with particle size less than 200 nm, are yielded by removing the solvent. The resulting dispersion is translucent and the typical diameter of the resulting paclitaxel nanoparticles is 140–160 nm. And an aseptic filter membrane (pore 220 nm) is usually used for sterilization by filtering out the impurity, bacteria, and etc. What is more, if necessary, ultrafiltration-technology can be applied for further purification to eliminate small contaminated molecules. In order to improve the stability of the products, lyophilization is always employed to prepare the solid powders of nab-paclitaxel (US20046749868, US20036537579, US20046753006, US20036537579, US20036506405, US5916596).

In addition to nab technology, desolvation (coacervation) and emulsification followed by fixation via chemicals (usually glutaraldehyde) or thermal treatment are also classic methods for albumin-based nanoparticle formation. In spite of these current loading trends, low entrapment efficiency and introduced toxicity are also predominant issues in solvent media preparation for these HSA nanoparticles (Yang et al., 2015). To overcome these problems, Gao et al. (2021) have explored a novel method. This method was based on two pivotal findings. The first one is that the solubility of paclitaxel in PEG can be significantly improved when ethanol is added

first to dissolve paclitaxel and PEG, after which the ethanol is removed. The other is that mixtures of paclitaxel in PEG and albumin powders can self-assemble into particles once they come into contact with water. Based on these findings, they dissolved paclitaxel and PEG in ethanol via ultrasound, and ethanol was subsequently evaporated under vacuum to obtain the liquid compound of paclitaxel and PEG. Then, paclitaxel was loaded in HSA powders by adding the liquid compound dropwise to HSA powder while stirring. Thereafter, the mixtures were added to phosphate-buffered saline (PBS) under ultrasound, after which nab paclitaxel suspensions emerged. PEG and the residual ethanol in suspensions were removed by dialysis and nab paclitaxel was finally obtained after freeze-drying.

#### 8.5.1.3 Microfluidic Methods

Microfluidic systems have entered the scene for the production of nanomedicines since last few decades (Convery and Gadegaard, 2019). There is a constant evolution of this technology. There is versatility in their mixing geometry and microchip design. The microfluidics technology is thus a very attractive and popular technique for nanoparticle manufacturing (Rezvantalab et al., 2018). These systems have the ability to simulate dynamic fluid flows and gradients. These have the ability to adapt to specific microenvironments. These can form multiorgan complexes, which provide an efficient and cost-effective approach for both NPs synthesis and screening. Microfluidic technologies allow for synthesis of NPs under controlled conditions. This can enhance batch-to-batch reproducibility. Microfluidic devices are versatile enough. So, it is possible to generate and customize endless platforms for rapid and efficient *in vitro* and *in vivo* screening of NPs' performance. Microfluidic devices are advanced systems that have great potential for small organism manipulation and immobilization. Gimondi et al. (2023) reviewed and summarize the major microfluidic platforms that allow for controlled NPs synthesis.

Microfluidics has great control over particle size and size distribution. It is working with low sample and reagent consumption. It has small physical and economic footprint. There is extreme sensitivity of these devices to streamline disturbances. There are possibilities of microchannel clogging and fouling phenomena, that can create a barrier for their industrial utilization. So, there is necessity of specialized microfabrication facilities. There is low-throughput due to slow flow (typically in the μL/min range). There are limitations of device compatibility with organic solvents (Lim et al., 2014; Min et al., 2014).

There is faster production rates using robust devices fabricated out of durable materials like polyimide-based, 3D hydrodynamic flow focusing microfluidic reactor. It has eight identical microchannels operating in parallel. It enabled the production of PLGA nanoparticles in ~10 g/h scale via nanoprecipitation (Min et al., 2014). Millifluidic systems can manipulate fluids in ~1 mm channels. These are shown to be suitable for large-scale production of PLGA nanoparticles. These devices include confined impinging jets mixers (CIJMs) and multi-inlet vortex mixers (MIVMs) and exploit the flash nanoprecipitation technique (Lim et al., 2014; Saad and Prud'homme, 2016; Pagels and Prud'homme, 2017; Sun et al., 2017; Turino et al., 2018). It uses rapid micromixing, on the order of milliseconds, to establish homogeneous supersaturation conditions and controlled precipitation.

### 8.5.2 Scale-Up Challenges in Nanomedicine Manufacturing

Manufacturing of nanomedicines under good manufacturing practices (GMP) conditions, as required by regulatory agencies, present challenges in scale-up (Desai, 2012; Sun and Yeo, 2012; Kaur et al., 2014; Agrahari and Hiremath, 2017; Souto et al., 2020). GMP guidelines describe minimum standards that manufacturers must meet in their production processes to ensure that the products are consistently of high quality, with batch-to-batch reproducibility. All steps in the manufacturing process need to be well documented. The final product needs to be appropriate for its intended use, and the medicine meets the requirements of marketing or clinical trial authorizations (EMA, 2018a).

Since a number of technologies and multiple unit-operations are often involved in nanomedicine production, the scale-up is critical. In downstream processes, process optimization and reproducibility can be achieved relatively easily at small scale. In preclinical studies and early clinical trials, nanoparticles obtained at small scales after well-characterization can be used as it usually require relatively small amount of product (Souto et al., 2020). In contrast, in large scale, it is challenging to achieve a robust manufacturing process for controlling the size and shape polydispersity of the nanoparticles. Challenges are also faced to achieve minimal batch-to-batch variations and maintaining the stability of the physicochemical properties of the product (Desai, 2012; Agrahari and Hiremath, 2017; Wu et al., 2020).

All the process-related variances have the potential to limit the clinical and/or commercial translation of nanoformulation. High degree of trial and error is required in process optimization for manufacturing a reproducible quality nanoparticle. This approach is ideally feasible for a laboratory setting. It is not commercially viable to perform trial and error for optimization of process in large-scale production. Generally, the cost of raw materials are very high, also there are additional need for an infinite number of multistep production processes, which make the industrial manufacturing of nanomedicines very costly in terms of time and money (Desai, 2012; Kaur et al., 2014; Agrahari and Hiremath, 2017; Wu et al., 2020).

It is required to consider early at the lab scale as what approach is suitable for scaling up the product. One should consider the manufacturing constraints in the industry to overcome the scale-up problems and save time. Establishment of predefined objectives regarding the quality target product profile (QTPP) and the critical quality attributes (CQAs) of the nanoformulation in order to achieve its safety and efficacy goals (Troiano et al., 2016; Colombo et al., 2018; Beg et al., 2019). Identification of QTPP and CQAs, such as particle size, size distribution, charge and morphology, and drug loading and release, impurity levels, etc., early in formulation design generally helps in establishing standards and determines whether or not a manufacturing process can provide batches that meet the batch-release criteria.

The impact of critical process parameters (CPPs) and critical material attributes (CMAs) on CQAs should be studied at development-stage only to ensure the quality of the finished product (Colombo et al., 2018). High product quality can be achieved by selecting suitable raw materials during formulation development, selecting adequate quality of the raw materials, and by adopting best production method that can preserve the physical and functional characteristics of the product, scale-up and

optimization of manufacturing processes both upstream and downstream (Namjoshi et al., 2020).

When evaluating CPPs and CMAs, it is often observed that some output variables (responses) are affected by multiple input variables (formulation and process parameters). For example, CMAs and CPPs for PLGA nanomedicine manufacturing involve (but are not limited to) type of PLGA, copolymer composition for polymer mixture and monomers ratio, APIs taken, organic solvent used, emulsifier/stabilizer, oil-to-water phase ratio, temperature, pressure, pH, process running time, and process volume. It is not possible to apply a single generic process to all nanoparticle preparations as each production technology has different operating parameters as well as each API and PLGA type have their own physicochemical peculiarities, and each nanosystem should be validated on a case-by-case basis (Agrahari and Agrahari, 2018). Therefore, analysis and characterization methods of nanoformulations are becoming more and more essential to validate the quality of the manufactured batches and to ensure that the CQAs are within the normal range and acceptance limits (Myerson et al., 2015). In order to determine the interaction of one and more input parameters and better understand the process, the design of experiments (DOE) approach is often exploited for product development, by most of the industries. DOE can help to provide maximal information from a minimal number of experiments, to evaluate the effects of CMAs and CPPs on the CQAs of the finished pharmaceutical dosage form (Cun et al., 2011; Namjoshi et al., 2020).

### 8.5.3 Scale-Up of Production of Nanoparticles

Although manufacturing of nanoparticles by different methods is clearly mastered at a lab-scale level as explained above, the transformation from the lab-scale production to commercial-scale production lack information in the literature concerning the manufacture of pharmaceutical grade nanoparticle suspensions. For instance,

- Nothing is known about the transposition of the lab production of doxorubicin-loaded poly(alkylcyanoacrylate) nanoparticles, Transdrug®, which are now produced in large quantities to provide with clinical batches used in the phase II/III clinical trials.
- This is also the case with the production of paclitaxel-loaded albumin nanoparticles, nab-paclitaxel or Abraxane®, used in clinics in the United States for the treatment of metastatic breast cancer.

Scale-up of methods for the production of pharmaceutical grade nanoparticle suspensions were only described for two types of methods: the emulsification–solvent diffusion method and the nanoprecipitation method. By extension, the scale-up approach developed for the method of emulsification–solvent diffusion was also applied to produce large batches of nanoparticles by the emulsification–reverse salting-out method. Going back to the classification of nanoparticle preparation methods proposed above, there are two methods based on two-step procedures requiring the formation of an emulsion, that is, emulsification–solvent diffusion,

emulsification–reverse salting-out, and one method classified in the one-step procedures, that is, nanoprecipitation.

These three methods were scaled up to a pilot-scale production, which is intermediate between the laboratory and the industrial production. Pilot-size production is aimed to simulate as close as possible the industrial production and hence needs to integrate all parameters that need to be optimized before reaching the industrial production.

#### 8.5.3.1 Pilot-Scale Production of Two Steps Procedures: Emulsification–Solvent Diffusion and Emulsification – Reverse Salting-Out Methods

To develop pilot production of nanoparticles by the emulsification–solvent diffusion method, it is required to set up a complete procedure including the preparation of saturated solvents. Table 8.6 summarizes the differences in the organization and equipment used in laboratory (volume of 60 mL) and pilot-scale production of the nanocapsules (volume of 2 L).

In the procedure, in one reservoir say Reservoir A is first used to prepare the mutually saturated solvents. Water and partially miscible organic solvents are mixed together to achieve the mass transfer process and reach the thermodynamic equilibrium of both liquids. Then, they are let to separate to give the two mutually saturated solvents required for this method. After phase separation, the lower phase, that is, water saturated with the organic solvent, is transferred in the reactor say B, through a valve say V1 while the organic solvent saturated with water is retained in the reactor A. Ingredients are then added in A and in B to complete the preparation of the organic and aqueous phases, respectively.

**TABLE 8.6**
**Comparison of the Equipment Used to Prepare Nanoparticles by the Emulsification–Solvent Diffusion Method at a Laboratory and Pilot-Scale Production (Colombo et al., 2001)**

| | Laboratory | | Pilot | |
|---|---|---|---|---|
| **Steps** | **Recipient** | **Agitation** | **Reactor** | **Agitation** |
| Saturation | Settling flask | Manual | Double-jacketed reactor (2–2.5 L) | Turbine, 4 paddles |
| Preparation of the aqueous phase | Round-bottom flask, (100 mL) | Magnetic | | |
| Preparation of the organic phase | Beaker (150–300 mL) | Turbine | | |
| Emulsification | | Ultraturrax T25 | | Ultraturrax T50 + Turbine, 4 paddles |
| Dilution | | Turbine | Reactor (6 L) | Turbine, 6 paddles |
| Purification | Evaporation under reduced pressure | – | Evaporation under reduced pressure | – |

*Source:* Colombo et al. (2001).

For instance, polymer, oil, and drugs are added in reactor A, while a surfactant is added in B. The organic phase prepared in A is then transferred in B by gravity through the valve V1 and the emulsion is prepared in B under vigorous stirring (Colombo et al., 2001). To obtain an emulsion with the desired characteristics, the design of reactor B and the mechanical mixer device were carefully optimized. The rationale behind this choice was to reproduce as much as possible fluid motions produced in the laboratory-scale setup production glass wares (Galindo-Rodriguez et al., 2005). Agitation for the emulsification is maintained over 30 minutes and the emulsion is then transferred through another valve say V2 in the reactor C containing the amount of water required for the dilution step. This reactor has the larger capacity, in the pilot plant (Colombo et al., 2001; Galindo-Rodriguez et al., 2005).

#### 8.5.3.2 Pilot-Scale Production of Nanoparticles by a One-Step Procedure Based on the Nanoprecipitation of a Polymer

In the nanoprecipitation method, the organic and the aqueous phases which are miscible are allowed to mix together producing the precipitation of the polymer as nanoparticles. In this method a critical point is the mixing of the two phases which is driven by the miscibility of the solvents. Thus, designing the pilot plant, efforts were stressed on the development of a specific mixing device allowing the two phases to come in contact and mix together with a continuous feeding process. This mixing device, which T shape was optimized, is the central piece of the pilot plant setup (Briançon et al., 1999; Galindo-Rodriguez et al., 2005).

The whole pilot plant includes a total of three reactors having equal capacities. The reactors A and B are filled out with the stock solutions of the aqueous and organic phases, respectively which are used to feed the T-shape mixing device at flow rates perfectly monitored under the control of peristaltic pumps. The outlet of the T-shaped mixing device is connected with reactor C used as a receiver of the nanoparticle suspension which actually formed in the T-shaped mixing device. In this configuration, the T-shaped mixing device can be continuously feed with the aqueous and organic phases insuring a continuous production of well-characterized nanoparticles. The obtained raw nanoparticle suspensions collected in the reactor C present good inter-batch reproducibility. The size of nanoparticle batches produced in the pilot plant can be up to 20-fold larger than batches produced in the laboratory scale (Galindo-Rodriguez et al., 2005; Tewa-Tagne et al., 2007). The only drawback inherent to the method is related to the low polymer concentration in the organic phase, which needs to remain in the dilute regime of the polymer solution (Legrand et al., 2007). This significantly limits the amount of nanoparticles recovered in the final raw dispersion.

### 8.5.4 Nanoparticle Purification and Collection Process

In lab-scale development, the purification of the particles is usually carried out using centrifugation and dialysis at the laboratory scale. However, centrifugation and dialysis are suitable to run in batch mode with the output of each step collected in a vessel. Also, these methods often require manual handling, which can be challenging, due to lack of reproducibility, particularly when increasing the scale to larger volumes (Dalwadi et al., 2005). All the unit operations need to be integrated

with the capability of recycling of streams and purging of impurities as required in order to perform the entire process in continuous mode (Rathore et al., 2015). In the panorama of continuous downstream process (CDSP) technologies for nanoparticle purification, the foremost exploited technologies, till date, are membrane separation techniques (tangential- or crossflow filtration) and continuous-flow centrifugation (Dalwadi et al., 2005; Holzer, 2017 (https://bioprocessintl.com/manufacturing/continuous-bioprocessing/continuous-downstream-processing-becoming-reality/); Łącki et al., 2018).

There are several suitable methods that can be applied to purify and collect nanoparticle or nanoparticle dispersions. They include:

1. Evaporation under reduced pressure
2. Centrifugation at low gravity
3. Ultracentrifugation techniques (Calvo et al., 1997; Govender et al., 1999; Lambert et al., 2000; Sahoo et al., 2002; Nguyen et al., 2003; Bouchemal et al., 2004, 2006)
4. Nano spray-drying
5. Filtration through mesh or filters (Govender et al., 1999; Murakami et al., 1999)
6. Ultrafiltration (Zahka and Mir, 1977; Allémann et al., 1993a)
7. Diafiltration (Tishchenko et al., 2001, 2003)
8. Crossflow microfiltration (Allémann et al., 1993b; Quintanar-Guerrero et al., 1998a; Limayem et al., 2004)
9. Dialysis (Chauvierre et al., 2003; Bravo-Osuna et al., 2006)
10. Gel filtration (Beck et al., 1990)

#### 8.5.4.1 Evaporation under Reduced Pressure

It is the most common approach to remove large quantities of volatile organic solvents and a part of water. This process is usually used after obtaining the nanoparticle suspensions by nanoprecipitation (Duclairoir et al., 1998; Peltonen et al., 2004; Zhang and Zhuo, 2005; Legrand et al., 2007), emulsification–reverse salting-out, emulsification–solvent diffusion (Guinebretière et al., 2002; Moinard-Chécot et al., 2008), and interfacial polycondensation combined with spontaneous emulsification (Bouchemal et al., 2004, 2006).

#### 8.5.4.2 Centrifugation

Centrifugation is the most commonly used unit operation for solid–liquid separation and is frequently exploited for nanoparticle purification, especially in lab-scale production. Tubular centrifuge is generally used in lab scale. Disc-stack centrifuge can run in both batch and continuous configurations (Tarleton and Wakeman, 2007). Centrifugal forces (usually up to 14,000 g) cause particles to accumulate on the underside of the centrifuge, where the particles slide to the outer periphery of the bowl automatically. It happens sometimes intermittently through nozzles located on the outer periphery of the bowl in a continuous manner (Tarleton and Wakeman, 2007; Rathore et al., 2015). Disc centrifuges are designed to accept wide range of feeds. But, they are mechanically complex and expensive. The complex geometry of

centrifuge also leads to difficulty in mechanical cleaning. In such cases, chemical cleaning must be used, which requires a more demanding QC process for cleaning validation (Tarleton and Wakeman, 2007).

Centrifugation at low speed (low gravity) can be applied to remove aggregates and large particles on most of the polymer nanoparticle suspensions. However, it does not eliminate all particles with a diameter above defined particle size as filtration on calibrated membrane does. Moreover, centrifugation at low gravity is not suitable to purify nanoparticles having a high density because they will sediment with aggregates. This happens in case of metal colloids type of nanoparticles. These are designed for applications in diagnosis by imaging techniques or in techniques based on thermal treatments applied in cancer therapy.

#### 8.5.4.3 Ultracentrifugation Methods

It consists of very high-speed centrifugations. Nanospheres, having a slightly higher density than water, can sediment and concentrate as pellets. These pellets are then separated from the dispersing medium found in the supernatant. Creaming occurs for oil-containing nanocapsules having a lower density than the aqueous dispersing media (Gallardo et al., 1993). In both cases, the dispersing medium containing all the unwanted residues can be removed and replaced by a new medium free from unwanted residues. Nanoparticles can be washed by several cycles of ultracentrifugation like two to three. After ultracentrifugation pellets or creams containing nanocapsules or nanospheres are dispersed in new dispersing medium (Peracchia et al., 1997; Duclairoir et al., 1998; Aboubakar et al., 1999; Kompella et al., 2001; Prabha et al., 2002; Bertholon et al., 2006; Legrand et al., 2007; Dreis et al., 2007; Hamoudeh et al., 2007). For most of nanospheres and oil containing nanocapsules developed so far as drug carriers, ultracentrifugations are performed at 100,000–110,000×*g* for 30–45 minutes.

The main problem of this technique is that nanospheres are not always easy to redisperse after ultracentrifugation (Pinto-Alphandary et al., 1995; Chiellini et al., 2003; Bertholon et al., 2006; Bouchemal et al., 2008). Aggregates may remain and the uses of vortex or ultrasounds are often mentioned as methods used to redispersed pellets after ultracentrifugation (Dreis et al., 2007; Hillaireau et al., 2007). Interestingly, Pinto-Alphandary et al. (1995) showed that purified nanospheres under a fully dispersed form can be collected after ultracentrifugation of the original suspension over a gradient of sucrose. The nanospheres concentrate in the gradient as a band of suspension in region of the same density than themselves while impurities remained in the upper phase of the lower density. Nanocapsules are more difficult to separate from the dispersing medium because the cream remains semiliquid. In addition, they are fragile and the application of several cycles of ultracentrifugation is hazardous because they can break easily (Quintanar-Guerrero et al., 1998a; Couvreur et al., 2002). Despite these drawbacks, ultracentrifugation appeared as a method of choice to facilitate the transfer of water-containing nanocapsules from the oil dispersion medium of their synthesis to an aqueous phase required for their *in vivo* administration by intravenous injections (Lambert et al., 2000; Hillaireau et al., 2007). In this case the ultracentrifugation is performed by overlaying the oil dispersion of nanocapsules on a cushion of an aqueous solution of surfactant. The

nanocapsules are transferred in the aqueous phase during the ultracentrifugation crossing the interface between the oil and the aqueous solution of surfactant. Then, they concentrate at the bottom of the ultracentrifuge tube to form a pellet. Although the method for the redispersion of the pellet obtained still needs to be improved, this is the only method that was found suitable to overcome this difficult task.

#### 8.5.4.4 Nano Spray-Drying

Nano spray-drying is atomization-based drying technology that enables the production of nanoparticles (Bohr et al., 2014). Although, these methods are commonly used for microparticle generation, nanometer-sized particles can also be achieved as demonstrated by Pamujula et al. (2004)) and Zarchi et al. (2015). Spray-drying is a scalable and rapid technique already established in pharmaceutical manufacturing for generating amorphous solid dispersion by uniformly dispersing APIs in a polymer matrix. Unfortunately, a low product yield is typically obtained due to loss of adhered product along the walls of the drying chamber. In addition, the production of particles at the nanometer scale, that is, size reduction is often limited due to insufficient liquid atomization forces (Pamujula et al., 2004; Bohr et al., 2014). The atomization force is contribution of centrifugal force and pressure of the airstream. Electrospraying has advantage of offering high EE and narrow particles size distribution, less agglomeration compared to other conventional atomizers, and offers various setup configurations for diverse purposes (e.g., single, coaxial dual or tricapillary nozzle). However, formulation optimization, and thus its application in large-scale production are limited or challenging due to large number of variable or adjustable parameters (Nguyen et al., 2016).

Spray-drying is one such technique that Sonaer Nozzles or particles generators, as well as "turn-key" spray-drying systems may be used for. Gelation and polymerization techniques are also used in conjunction with spray particle synthesis. These types of procedures are done by using Sonaer nozzles combined with dual microfeed (DMF) liquid feeds of two different liquids. For example, two polymer precursors may polycondense, and form gel-able polymer. A crosslinker, a monomer or oligomer precursor and initiator are sprayed using multiple hypo-needle feed attachment. Spray shaping attachments are utilized to help drying of microcapsules. It diverts gasses to nanoparticle formation or any associated reaction(s). It also focus and direct microcapsule precursors to a desired area for drying.

Sonaer has a line of micro feed tubes for controlling the flow of solution. The tubes are excellent for lower flow rates. The dual micro feed tube is a good choice for microencapsulation. This prevents two reactive solutions from contact until it reaches the nozzle tip prior to atomization.

Different particle structures and morphologies are often sought as well. Such can be produced by spray-drying or by spraying into a specifically chosen solution or reactive medium. Sonaer manufactures particle generators, nebulizers, and nozzles that can be incorporated into spray-drying systems, as well as full "turn-key" bench top-spray dryers. Another example process for producing micro- or nanoparticles is spray particle synthesis. Spray particle synthesis is a technique in which a liquid precursor or solution is sprayed into a medium of excess liquid or gas/vapor, where it then reacts to form small particles of a desired material.

Such a technique could be useful in micro- or nanoparticle or powder synthesis, as well as particle microencapsulation. By tuning the spray liquid concentration and spray droplet size produced, one may be able to tune the size of particles produced using this technique. Using a multiple hypo-needle attachment and/or DMF tube, one can potentially synthesize gel, polymerized, or multi-material microspheres (such as "janus"/"dimer" microspheres or core-shell particles).

Microspheres for pharmaceutical, medical, and consumer products are made with ultrasonic atomizer nozzles and equipment supplied by Sonaer. Suspensions, slurries, and other solution are made into fine misty spray for fabricating microspheres. Microspheres are then collected and incorporated into toothpaste, medicine, implants, fuels, and other products requiring uniform spheres.

#### 8.5.4.5 Filtrations through Mesh or Filters

These are applied to remove large particles or polymer aggregates which formed during preparations (Murakami et al., 1999; Govender et al., 1999). Such purification is systematically applied on nanoparticle suspensions designed for intravenous injections. Although these methods show many advantages, caking is a common problem found when a colloidal suspension is pressed through a filtration membrane. This corresponds to the formation of a precipitate of particles on the filtration membrane surface. If the adhesion of the particles is stronger than the repulsion, the cake formed is irreversible and membrane permeability decreases dramatically (Harmant et al., 1996).

There are alternate filtration methods suggested based on different principles, like crossflow filtration, diafiltration, ultrafiltration, and microfiltration (Quintanar et al., 1999; Tishchenko et al., 2001; Limayem et al., 2004; Dalwadi et al., 2005; Bouchemal et al., 2008).

##### *8.5.4.5.1 Crossflow Filtration*

Batches of nanoparticles can be purified completely in less than 3 hours by crossflow filtration using a microfiltration membrane (Allémann et al., 1993 a, 1993b). The method of crossflow filtration can also be applied with ultrafiltration membrane and it generally requires only very little manipulations. In addition to their rapidity, these purification procedures are more efficient than dialysis and can be applied with minimal detrimental impact on nanoparticle size and drug-loading capacity (Limayem et al., 2004). The cake formation can be reduced by using the crossflow filtration mode, by using hydrophilic membranes (Madaeni and Fane, 1996), or by using diafiltration with suction (Tishchenko et al., 2003). The purification of nanoparticle suspensions by crossflow microfiltration was investigated at a large scale (Quintanar-Guerrero et al., 1998b; Quintanar et al., 1999).

##### *8.5.4.5.2 Diaflitration*

The process involves two steps, a concentration step in which the nanoparticle suspension is concentrated by a factor of 1/5, followed by a diafiltration step in which the volume of the feed suspension is kept constant by continuous addition of pure water (Limayem et al., 2004). Among all the purifications methods based on crossflow filtration and diafiltration present many advantages. They combine a high efficacy

with the possibility to operate a concentration of the suspension without causing aggregation of the nanoparticles. Moreover, these methods are applicable in large-scale productions suitable for industrial developments.

#### *8.5.4.5.3 Tangential Flow Filtration (TFF)*

Tangential flow filtration (TFF) is a type of filtration that can run continuously where the fluid flows parallel to the filter membrane, along the surface of a filter membrane rather than passing through it. The main advantage is that the filter cake does not settle on the filter, but rather is flushed away during the process, increasing the length of time a filter unit can be operational (Łącki et al., 2018). This method can efficiently remove the stabilizer molecules (approximately 91%), with low impact on yield, size, and stability of the finished product. Scale-up of a TFF is possible and easier, since the membrane cartridges (cassettes or hollow fibers) are linearly scalable. This process is scalable linearly, that can be achieved by using geometrically similar membrane cartridges at different scales. Geometric similarity ensures scaling up, scaling down, and for process validation purposes. This can be achieved, without compromising on process performance, by keeping the processed volume per membrane area constant at different scales (Łącki et al., 2018). An increase in the viscosity of the permeate may cause reduction in transmembrane pressure (the pressure difference between the two sides of the membrane) and diminish the filtration efficiency. This can be time consuming for large-scale manufacturing processes (Łącki et al., 2018).

TFF cartridges are generally made of membranes like regenerated cellulose (RC), cellulose ester (CE), mixed cellulose (ME), polysulfone (PS), polyethersulfone (PES). These membranes generally are associated with problems like adsorption of particles, as well as presence of biological elements in the treated specimens. To solve these problems, modified PES (mPES) materials were developed, which are more hydrophilic and can reduce fouling (or bio-fouling) phenomena (Raghunath et al., 2012).

Since most of the polymers need dissolution in organic solvents such as dichloromethane, ethyl acetate, dimethyl sulfoxide, etc., compatibility of solvent with membrane filters must be evaluated carefully. For example, regenerated cellulose cartridges are recommended for purification of nanoformulations containing dichloromethane. Nevertheless, appropriate dilutions prior to running the TFF process along with pre-assessment trials are recommended since variations in temperature, concentrations, durations of exposure, and other factors may affect the performance of the membranes. In addition, when choosing an appropriate fiber filter, the molecular weight cut-off of the membrane as well as total membrane surface area should be considered respectively, depending on the size of the particles and the sample volume to be processed (Raghunath et al., 2012).

### 8.5.4.6 Purification by Dialysis

This can be performed using different kinds of cellulose membranes of various molecular weight cut off allowing substances having low or high molecular weight to diffuse toward the counter dialyzing medium thanks to the concentration gradient of solutes. Although dialysis is a simple and common method used to purify

nanoparticles, premature release of nanoparticle payload can occur during the long purification period it requires and, because large volume of counter dialyzing medium are required to make the purification efficient. Furthermore, the application of dialysis in a large scale is disputable from an economical point of view and from the high risk of microbial contamination of the final product due to the long duration of the process.

#### 8.5.4.7 Gel Filtration

As another purification process, gel filtration can be used (Beck et al., 1990). It is also much faster than methods based on simple dialysis, but it is greatly limited by the relatively small volume of sample which can be processed at a time. In addition, irreversible adsorption of actives onto the column stationary phase and the poor resolution between large impurities and small nanoparticles can restrict the use of this technique for purification of drug-loaded nanoparticulate formulations.

### 8.5.5 Drying and Storage Process

Physicochemical integrity of nanomedicine must be preserved over time throughout its shelf-life, in addition to the degree of purity. Like all pharmaceutical products, during storage nanomedicine can undergo degradation by various pathways, mediated by water, air (oxygen), light and heat, which can lead to lower potency or even toxicity of the formulation. Research has been shown that hydrolytic degradation of PLGA particles in aqueous media can take place as early as 15 days depending on the PLGA molecular weight, lactide:glycolide ratio and particle morphology (Keles et al., 2015; Swider et al., 2019). This may be due to the presence of water. Therefore, an efficient removal of water content is ideally needed to improve the nanomedicine stability. Generally drying of such nanoparticles improves stability, as well as to facilitate handling, reduces storage space and transportation costs (https://www.lyophilizationworld.com/post/spray-drying-as-alternative-to-lyophilization-in-the-pharmaceutical-industry dated 02 Nov. 23).

Lyophilization and spray-drying are the most commonly used techniques to dry nanoformulations to improve stability. Continuous aseptic spray-drying (Adali et al., 2020), and continuous freeze-drying (Van Bockstal et al., 2017a, 2017b) can both potentially be exploited for continuous manufacturing of nanomedicine in future. Continuous freeze-drying utilizes infrared radiation for drying the frozen samples in spinning vials. This can improve process efficiency and product quality (uniformity) compared to conventional batch freeze-drying processes.

#### 8.5.5.1 Drying of Nanoparticles

As in the case of many pharmaceutical preparations, storage of nanoparticles as suspensions presents many disadvantages such as risk of microbiological contamination, premature polymer degradation by hydrolysis, physicochemical instability due to particle aggregation and sedimentation, and loss of the biological activity of the drug (Magenheim and Benita, 1991). To circumvent such problems, pharmaceutical preparations are stored under a dry form. In general, the transformation into a dry

product can be achieved using freeze-drying of a liquid preparation (Abdelwahed et al., 2006) or spray-drying processes (Tewa-Tagne et al., 2006, 2007).

#### *8.5.5.1.1 Freeze-Drying of Nanoparticles*

Freeze-drying, also known as lyophilization, is a very common technique of conservation used to ensure long-term stability of pharmaceutical and biological products preserving their original properties (Layre et al., 2006). A review on the application of the freeze-drying process to nanoparticle suspensions was published by Abdelwahed et al. (2006). The basic principle of this process consists of removing water content of a frozen sample by sublimation and desorption under vacuum. In general, freeze-drying processes can be divided in three steps: freezing of the sample (solidification), primary drying corresponding to the ice sublimation and secondary drying corresponding to desorption of unfrozen water. In the case of nanoparticle suspensions, the cryo-concentrated phase include nanoparticles, remaining free surfactants, buffer, and free drugs. During the freeze-drying process, several problems may arise which can lead to a loss of integrity of the nanoparticle characteristics. For instance, crystallization of ice may exert a mechanical stress on nanoparticles leading to their destabilization. This effect is more critical during the lyophilization of nanocapsules which are very fragile upon lyophilization (Abdelwahed et al., 2006).

The high concentration in nanoparticles in the final dried product may favor aggregation and even in some cases irreversible coalescence of nanoparticles. The addition of cryoprotectants can improve the resistance of nanoparticles toward freezing and drying stresses and also increase stability during long-term storage. In general, the type of cryoprotectant is selected in the aim to ensure a maximum stabilization of nanoparticles. Sugars including trehalose, mannose, sucrose, glucose, lactose, maltose, and mannitol are often used but the level of stabilization generally depends on their concentrations (Chasteigner et al., 1995; Jaeghere et al., 1999; Sameti et al., 2003; Abdelwahed et al., 2006).

The weight ratio cryoprotectant/nanoparticles is an important parameter to consider to preserve the stability of the nanoparticles during freeze-drying. For instance, weight ratio trehalose/nanoparticles (1/1) was found as optimal to promote the complete redispersion of freeze-dried PLA-PEG nanoparticles (Jaeghere et al., 1999). In some cases, increasing cryoprotectant concentration above the optimal value can compromise the stability of the nanoparticles and even promote their destabilization (Sameti et al., 2003). Although sugars are the most popular cryoprotectants, other components can protect nanoparticles during lyophilization.

For instance, PCL nanocapsules remained intact after lyophilization in the presence of an excess of PVA added to the preparation medium at concentrations ranging from 2.5% to 5% (Abdelwahed et al., 2006). Similarly, poly(isobutylcyanoacrylate) and poly(isohexylcyanoacrylate) nanoparticles can be freeze-dried without any modification of their size in the presence of 2% of pluronic® F68 (Seijo et al., 1990). Besides excipients, the freeze-drying process parameters can impact the texture of the frozen matrix and the final morphological characteristics of the freeze-dried cake. In general, the optimization of freeze-drying cycle is aimed to shorten the sublimation duration which is the longest step of the whole process (Brigger et al., 2003; Abdelwahed et al., 2006). Finally, freeze-dried nanocapsules should be stored at a

temperature below the glass transition temperature of the formulation to maintain the glassy state of cryoprotectant and to prevent aggregation of the nanocapsules. This was the case of freeze-dried nanocapsules made of poly(vinylpyrrolidone) (PVP) which remained stable after six months of storage under accelerated stability conditions (40°C) (Abdelwahed et al., 2006).

#### *8.5.5.1.2 Spray-Drying*

The spray-drying technique transforms liquids into dried particles under a continuous process. It might be an interesting alternative to freeze-drying thanks to advantages like low price, rapid process, and possibility to modulate physicochemical characteristics of the produced powders by varying process parameters (Broadhead et al., 1992). It is one treatment which suits with heat-sensitive molecules like proteins preserving them from significant degradation (Adler et al., 2000). Nanoparticle formulations submitted to spray-drying are generally aqueous suspensions and contain one soluble compound added as drying auxiliary. Examples of drying auxiliaries are colloidal silicon dioxide (Müller et al., 2000; Tewa-Tagne et al., 2006), lactose, mannitol, and PVP (Tewa-Tagne et al., 2007). Spray-drying process includes four important steps: (1) atomization of the feed, that is, nanoparticle suspension, into a spray, (2) spray-air contact, (3) drying of the spray, and (4) separation of the dried product from the drying gas (Master, 1991). Similar to freeze-drying techniques, the nanoparticle suspension is converted into a dry formulation. The powder form should be stored at temperature under the glass transition temperature of the polymer forming the nanoparticles. The main problem can be found during redispersion of the powder where aggregates may be difficult to dissociate.

### 8.5.6 Nanoparticle Sterilization Approaches

Lastly, for systems intended for parenteral administration, finding an appropriate sterilization method is a crucial final step in manufacturing. Each preparation must be validated on a case-by-case basis as nanoparticles can be affected differently by the sterilization method, depending on their components, formulation, and/or method of preparation (Ragelle et al., 2017).

Sterilization procedures such as gamma irradiation or autoclaving can be detrimental to sensitive APIs, cause PLGA chain alteration, and affect the overall characteristics of the nanoformulation itself (Bozdag et al., 2005; Keles et al., 2015). In this case an alternative method can be sterile filtration of the final product. Nevertheless, this process only works for nanoformulations with a size distribution smaller than 0.22 µm.

Finally, aseptic manufacturing can be implemented. However, aseptic processes can be costly and difficult to perform particularly for multistep processes (Kaur et al., 2014; Ragelle et al., 2017). Among the described production technologies, aseptic manufacturing is the one that best lends itself to continuous production processes, where production is totally isolated from the surrounding environment and operators. Once established, the conditions needed for sterile continuous production can be maintained indefinitely, for days, weeks, even months (https://www.pharmtech.com/view/potential-continuous-sterile-manufacturing dated 02 Nov. 2023).

## 8.6 ANALYTICAL CHARACTERIZATION OF NANOSUSPENSIONS

Since every nanoparticle is distinct and unique, basic characterization of nanoparticles is done by particle size distribution, zeta potential measurement, optical microscopy, and solid-state characterization.

### 8.6.1 Particle Size Distribution

Based on the angular intensity of scattered light in a sample, laser diffraction instruments measure particle size. Two light sources (laser and visible lamp) are directed in an optical cell, through which sample is dispersed in an aqueous medium and circulated. Multiple detectors measure the intensity of light, at various scattering angles, which is used to calculate particle size distribution, mean, and median for any nanosuspension.

### 8.6.2 Zeta Potential

Zeta potential measurement provides valuable information for the surface properties of nanoparticles. This is especially useful, when ionic surfactants are used to stabilize a formulation. To impart enough electrostatic repulsion and provide a stable nanosuspension, usually, zeta potentials greater than +40 mV or less than –40 mV are sufficient.

### 8.6.3 Optical Microscopy

Nanoparticles cannot be seen with optical microscopy, but it is a valuable characterization tool for nanosuspensions. Nanoparticles may appear as vibrating points within a suspension due to sufficient thermal energy under optical microscopy, to keep the particles discrete and in continual motion. This indicates that the particles are not aggregating. Since, this reaches a lower-energy state and the nanosuspension is physically stable.

Microscopy allows one to see large aggregates or single API crystals that have grown through Ostwald ripening. If a nanosuspension is being developed for parenteral administration, it is important that these large particles are identified.

### 8.6.4 Solid-State Characterization

Methods include x-ray powder diffraction, differential scanning calorimetry, FTIR spectroscopy, and Raman spectroscopy which are used to assess and monitor the solid-state properties of the API during the milling process. Most of the cases, there is no change in morphology, but one can't assume this will be always the case and should generate data during the development process.

## 8.7 NEW COMMERCIAL TECHNOLOGIES IN NANOPARTICLE PRODUCTION

### 8.7.1 Seamless Scaling for Nanomedicine Development

Nanomedicines represent the next paradigm in drug innovation with treatment modalities like gene therapy and targeted medicine, offering improved efficacy and reduced side effects. Nanoparticle formulations vary in particle size, payload and

excipient characteristics opening up possibilities for difficult-to-deliver drugs and hard-to-treat targets (NxGen – A Disruptive Technology Enabling Transformative Medicine (precisionnanosystems.com) dated 30 Oct. 2023). However, taking nanoparticles from research to market is a challenging activity, specifically manufacturing nanoparticles with reproducible desired properties. Along with new formulation development, process development is also a global requirement. Many companies are working on developing manufacturing devices to achieve reproducible and precise nanoparticles following cGMP requirements (https://www.precisionnanosystems.com/platform-technologies/nxgen accessed on 27 Nov. 2023).

Precision NanoSystems is a global leader in nanoparticle technologies, solutions, and services for developing genomic medicines, including mRNA vaccines and therapeutics that define the future of medicine.

- NanoAssemblr® instruments with proprietary NxGen™ microfluidic mixers with scalable flow rates enable increased nanoparticle stability, efficacy, yield and quality, accelerating timelines to develop robust and reproducible formulations from discovery to commercial manufacturing.
- GenVoy-ILM™ off-the-shelf Research Use Only (RUO) LNP delivery reagents accelerate discovery and proof-of-concept studies to clinical development. GenVoy-ILM mRNA-LNP showed similar biophysical and biological behavior when compared to mRNA-LNP containing the ionizable cationic lipid, MC3, which is used in the FDA-approved RNA-LNP Onpattro®.

Vaccines, gene therapy, and cell therapy demand different characteristics from lipid nanoparticles. For example, mRNA-LNP vaccines are administered by intramuscular injection and are designed to elicit an immune response. In contrast, mRNA-LNP is administered by intravenous infusion and formulations that provoke an immune response can affect therapeutic efficacy. Precision Nanosystems' LNP library comprises proprietary lipids and LNP formulations that have been designed for specific applications.

Precision NanoSystems enables the world's leading drug developers to create genomic medicines by offering NanoAssemblr® instruments, ionizable lipids, off-the-shelf reagents, and BioPharma services. Their LNP portfolio provides access to off-the-shelf kits and custom formulations for developing nucleic acid therapeutics that seamlessly transition from discovery to the clinic. Rationally designed LNP formulations are used for generating proof-of-concept and efficacy data. This allows progression of lead candidates quickly through screening and *in vivo* preclinical studies to accelerate clinical evaluation (https://www.precisionnanosystems.com/platform-technologies/genvoy-platform/Lipid-Nanoparticle-Portfolio accessed on 27 Nov. 2023).

In genomic medicines, the drug delivery challenge is getting the API into the target cells at the disease site. LNPs are an elegant solution for DNA or RNA delivery as they protect the cargo from degradation and mediate a safe and targeted intracellular delivery. LNPs consist of ionizable cationic lipids, helper lipids, cholesterol, and stabilizers, each having a specific role in delivery of the genomic medicine to its site to activation. Due to their modular nature, excipients can be mixed, matched,

and modified during LNP formulation to target different organs in the body and improve the encapsulated drug's efficacy and pharmacokinetic profile.

In particular, ionizable lipids offer a unique capability to shift charge with pH changes to ensure strong binding of nucleic acids during LNP formulation while maintaining a neutral charge under physiological pH to limit cytotoxic effects. They are the main component that enable efficient cellular delivery and overcome payload size limitations. Access to an LNP portfolio with an array of ionizable cationic lipids allows for LNP optimization for targeted nanomedicines. In addition to liposomes and polymeric nanoparticles (poly(lactic-co-glycolic acid) PLGA) nanomedicines, LNP delivery technology is clinically proven with mRNA delivery for gene therapy through Onpattro, COVID-19 vaccines (https://www.nature.com/articles/s41578-021-00358-0 accessed on 27 Nov. 2023) and is currently in clinical trials for several cell therapy applications including CRISPR-Cas9 gene editing, protein replacement therapy, and more. Precision NanoSystems offers an easily accessible and well-characterized LNP portfolio and nanomedicine formulation technologies for these applications and future RNA-based therapeutics.

Alterations in the chemical structure and ratio of the LNP components affect its delivery efficiency, potency, and biodistribution, making it essential to optimize LNPs for each application so that it is fit-for-purpose. LNP formulations are not one-size-fits-all, as ionizable cationic lipids that are effective for mRNA vaccines may not be well-suited for gene editing or cell therapies. Aside from safety and potency, optimizing ionizable lipids for structural properties, targeted RNA delivery and immunomodulation can be labor-intensive. Access to an LNP portfolio that includes systematic categorization of these lipids allows for rationally designed custom LNP formulations that help developers quickly reach the full potential of nucleic acid therapeutics across applications.

Their end-to-end Genomic Medicine Tool Kit uniquely enables access to ionizable lipid mixes through the LNP portfolio, LNP technologies and proven expertise, to establish robust in-house strategies and infrastructure (Figure 8.6). This results in a sustainable LNP formulation and manufacturing framework that accelerates drug candidates across indications from discovery to the clinic.

Nucleic acid therapeutics including mRNA-LNP vaccines, gene therapy, cell therapy, and more, demand different characteristics and indications from LNPs, requiring fit-for-purpose optimization to produce effective nanomedicines. To accelerate development, the LNP portfolio offers off-the-shelf LNP formulations designed for specific applications with a clear path to the clinic, and proven expertise in biopharma

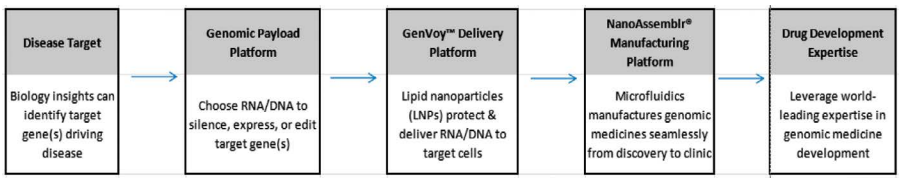



**FIGURE 8.6** End-to-end Genomic Medicine Tool Kit (https://precision.opacity.design/workflows/genomic-medicine accessed on 9 Jan. 2025)

services to help in selecting appropriate ionizable cationic lipids for custom applications. Their easy-to-use kits or custom formulations enable any researcher to become a genomic medicine developer as they offer ionizable lipids for broad or targeted applications. This saves significant time for validation of target payloads and lowers the risk of developing a bespoke LNP formulation, accelerating preclinical programs for lipid-based therapeutics.

Nanomedicine developers must successfully file regulatory documentation before conducting clinical trials on cutting-edge novel medicines. Therefore, submitting an Investigational New Drug (IND) application for RNA therapeutics to the FDA is a significant milestone. For a successful IND filing, it is possible to prepare for scale-up and manufacturing processes during each development phase to streamline the overall process. Starting with the end in mind, and identifying clinical goals at the outset allows researchers to establish preclinical programs that generate supporting data and manufacturing strategies at each stage, strengthening the IND filing and accelerating the path to clinical development. Nanomedicines represent the next paradigm in drug innovation with lipid-based nanoparticles offering revolutionary new treatment modalities including saRNA and mRNA-LNP vaccines, gene therapy, and cell therapy based on LNP delivery technology.

#### 8.7.1.1 Advanced Technology for Advanced Genetic Medicines

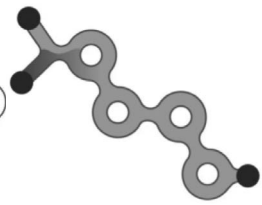
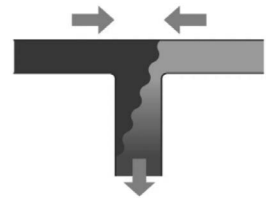
NxGen technology preserves non-turbulent mixing conditions to provide unparalleled control over nanoparticle assembly that is scalable from discovery to the clinic. (https://www.cytivalifesciences.com/en/us/knowledge-center/explore-nanoparticle-types-properties-applications?srsltid=AfmBOop8sbwlmWNreRY63thNfYp5uirKQoULCkW7uUv57bOfcaUd2NY4 accessed on 09 Jan. 2025)

##### *8.7.1.1.1 How Lipid Nanoparticles Are Formulated for Clinical Use*

**Basic Principles**

1. An aqueous phase and a miscible solvent containing dissolved nanoparticle precursors are injected into each inlet of the NanoAssemblr cartridge. An organic solvent containing dissolved lipids and an aqueous solution containing nucleic acids are injected into the two inlet channels of the NxGen™ cartridge.
2. Under laminar flow, the two phases do not immediately mix (Table 8.7).
3. Microscopic features engineered into the channel reproducibly control the mixing of the two streams.
4. Rapid, controlled, homogeneous mixing produces homogeneous nanoparticles. Within millisecond, the two fluids are completely mixed, causing a change in solvent polarity that triggers the self-assembly of LNPs loaded with nucleic acids.
5. Changing the speed and ratio of fluid injection control the size of the LNPs. Once manufactured, LNPs mimic low-density lipoproteins in the body. These structures allow them to be taken up by an endogenous cellular transport pathway to deliver nucleic acids to cells. Using pH-sensitive lipids allows LNP to release encapsulated nucleic acids into the cytoplasm when vesicle pH decreases.

**TABLE 8.7**
**Comparison of the NanoAssemblr™ Classic Mixer (SHM), NxGen™ Mixer (Toroidal Mixer (TrM)), and T-Junction Mixer**

| NanoAssemblr™ Classic Mixer | NanoAssemblr™ NxGen™ Mixer | T-Junction Mixer |
|---|---|---|
| |  |  |
| Flow rate capacity: 1–20 mL/min<br>Nominal gross product capacity: >850 mg (>28,000 vaccine doses) per mixer per 4-hour shift<br>Platform used:<br>NanoAssemblr™ classic mixer | Flow rate capacity: 1 to >200 mL/min<br>Nominal gross product capacity: 8.5 g (>283,000 vaccine doses) per mixer per 4-hour shift<br>Platform used:<br>NanoAssemblr™ Spark™ system<br>NanoAssemblr™ Ignite™ and Ignite+™ systems<br>NanoAssemblr™ Blaze™ system<br>NanoAssemblr™ GMP system | Flow rate capacity: 40–60 mL/min<br>Nominal gross product capacity: 2.6 g (85,000 vaccine doses) per mixer per 4-hour shift (based on typical mRNA formulation parameters and vaccine dose of 30-µg mRNA per dose). Does not account for process yield |

*Source:* https://www.cytivalifesciences.com/en/us/knowledge-center/microfluidic-technology-for-scaling-nanoparticle-production?srsltid=AfmBOoqiiIV8KLx22ee1PCVt6NPLGrItmSQ9IAqCCUZqL-yp5CQLPGTv accessed on 09 Jan. 2025

**Non-turbulent mixing offers advantages over traditional formulation methods** (https://www.cytivalifesciences.com/en/us/knowledge-center/microfluidic-technology-for-scaling-nanoparticle-production?srsltid=AfmBOoqiiIV8KLx22ee1PCVt6NPLGrItmSQ9IAqCCUZqL-yp5CQLPGTv accessed on 09 Jan. 2025):

1. Uniform-sized nanoparticles of around 50–300 nm, due to control the flow and mixing conditions.
2. Better drug-loading efficiencies and storage.
3. Promising delivery results for clinical applications help protecting nucleic acids to help maintain their stability.
4. Translation of liposomes from the bench to the clinic with good scalability and liposome formulations has high protein loading (20% to 35%) compared to sonication or extrusion methods (<5%).
5. Optimized production led to the generation of highly loaded liposomes with size control (60–100 nm) and a polydispersity index (PDI) <0.2.

### 8.7.1.2 Drug Discovery and Screening with NanoAssemblr® Spark™

The Spark instrument overcomes the challenges of traditional techniques for the controlled and reproducible manufacturing of LNPs at low volumes. It can also help

**FIGURE 8.7** NanoAssemblr tools.

establish CQAs, such as cargo sizing and the ratio between the positively charged lipid amine groups (N) and the negatively charged nucleic acid phosphate groups (P) or N/P ratio.

In a recent study, Precision NanoSystems created an mRNA-LNP that encodes for the EPO gene, a potential model for the treatment of patients with anemia, as RNA and LNP delivery systems can eliminate the need for cell culture, making therapeutic development easier. The EPO RNA-LNPs were scaled up using the NanoAssemblr instruments (Figure 8.7) (Ignite, Blaze and GMP System) and CQAs were examined to quantify particles across platforms in terms of size, polydispersity, and EE. No significant difference was found between the lab and clinical manufacturing scales. Further, evaluation of *in vivo* data showed that hematocrit and serum EPO levels are consistent across batches indicating that particles have the same biological effect *in vivo* regardless of the manufacturing platform.

This study data validates that Precision NanoSystems' technologies enable developers to de-risk the scaling process by ensuring consistent CQAs of LNPs as they scale from the lab to larger scales.

The NanoAssemblr® Spark is designed to produce microliter volumes (25 to 250 µL) nanomedicine formulations. Advanced microfluidics is helpful in formulating microliter formulation volumes with near-complete sample recovery. Simply pipette starting materials into wells, push a button, and pipette completed formulations out. The Spark formulation process requires less than 5 seconds, allowing hundreds of formulations to be made in hours. The Spark system is designed for operation in a sterile biosafety hood, so formulations can be made on-demand, and applied to cells in culture. Shared microfluidic mixing technology allows formulations to be scaled across the NanoAssemblr Platform to accelerate future development. Neuro9 kits contain everything you need to encapsulate siRNA into clinically validated LNP delivery systems. Kits include a proprietary ionizable cationic lipid.

**The NanoAssemblr® Ignite™:** Advanced microfluidics in an automated system removes batch-to-batch and user-to-user variability. Easy setup with touch screen interface, saved recipes, and a single-use fluid path that eliminates priming and cleaning enables a streamlined workflow. Tune particle size with precise control over fluid flow rates. Formulate small molecules, peptides, and nucleic acids into lipid, polymer or hybrid nanoparticles, and more. Formulation runs require less than

a minute. Over 50 formulations can be completed in a day for rapid optimization. Easily scale between 1 mL and 20 mL formulations on the Ignite™. Optimized formulations can be predictably scaled to advanced preclinical and clinical scale with Blaze™ and GMP Systems with NxGen technology.

**The NanoAssemblr® Blaze:** Efficient formulation for large preclinical studies, with single-mixer flow rate up to 115 mL/min with NxGen technology. De-risk processes by using the same NxGen microfluidic mixer on Blaze and GMP. Formulation of 10 mL to 1 L batches with the Blaze or up to 10 L with the Blaze+. Optional integration to up- and downstream processes and continuous pressure monitoring. Validation of inline dilution. The signature simplicity and efficiency of the NanoAssemblr platform.

**GMP System (Figure 8.8): Power to Commercialize:** The NanoAssemblr GMP System is a modular and configurable system, fit-for-purpose, and phase appropriate for the GMP manufacturing of genetic medicine.

The GMP system is a continuous-flow microfluidic platform designed for scale-up manufacturing of nanoparticles under cGMP conditions. The formulation results are in sync with NanoAssemblr Ignite and Blaze-generated particles.

Precision NanoSystems also provides services for fast nanoparticle development, like Ignite and Ignite+ that undertake optimization of crucial factors at initial stages and with the NanoAssemblr Blaze and Blaze+, wherein the process development

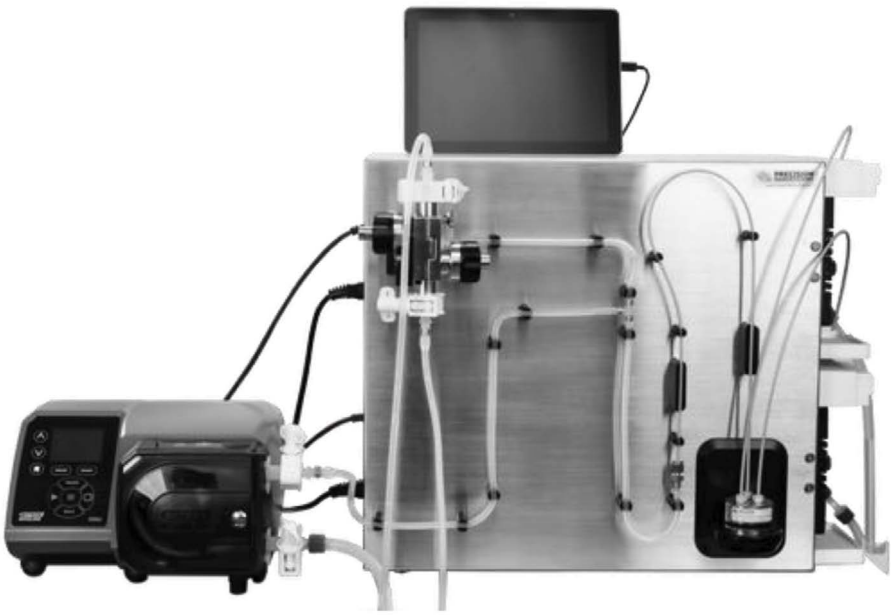


**FIGURE 8.8** GMP system of NexGen technology for nanoparticle production. (From https://www.cytivalifesciences.com/en/us/shop/lipid-nanoparticle-instruments-and-reagents/nanoparticle-formulation-cartridges-and-flow-kits/nanoassemblr-gmp-system-fluid-paths-p-45325?srsltid=AfmBOoqaFeGe738EjbNJ6gNvnGWSs8d1f2jyPAxB1KDQZGHWeiJZ1nW9 accessed on 13 Jan. 2025.)

can be conducted on both the upstream and downstream portions. This streamlined development from material preparation to buffer exchange, filtering, and analytics ensures that a program is ready to quickly accelerate toward the clinic. From discovery to clinic, the NxGen architecture produces uniform and reproducible high-quality LNPs, reducing the risk of scaling processes. The CPPs involved in this equipment are flow rate ratio and total flow rate.

#### 8.7.1.3 Develop a Diverse Range of Formulations

Polymer nanoparticles, liposomes, and nucleic acid LNPs can be optimized. For example:

1. PLGA nanoparticles were optimized by systematically varying the initial polymer concentration. PLGA was dissolved in acetonitrile and mixed at a flow rate ratio of 1:1 with water containing 2% PVA.
2. Liposome formulations were optimized by systematically varying the flow rate ratio. Lipids were dissolved in ethanol and mixed at different flow rate ratios with PBS buffer.

Statistically equivalent results were obtained between Classic and NxGen configurations at the bench scale.

#### 8.7.1.4 Critical Process Parameters (CPPs) Maintained from Ignite to GMP System

Downstream processing time, flow rate ratio, total flow rate, and TFF are critical parameters during scale-up. The Ignite+ can manufacture RNA-LNPs at flow rates of up to 200 mL/min and batch sizes of up to 60 mL. This capacity allows for small-scale evaluation of RNA-LNPs using the same CPPs used in large-scale cGMP production. Late preclinical studies can be conducted efficiently with the NanoAssemblr Blaze using a process that mimics clinical scale implementation. Furthermore, the Blaze+ upgrade enables the formulation of volumes ranging from 20 mL to 10 L, allowing for late preclinical experiments and process development activities (downstream filtration and analytical protocols).

CPPs for the TFF process, such as filter material and construction, shear rate and target transmembrane pressure, must all be determined and optimized for LNP formulations as the transition to TFF is an essential activity and should be de-risked early in process development.

When developing an RNA-LNP COVID-19 vaccine, Precision NanoSystems demonstrated the significance of early testing of formulations for downstream process parameters. An important step for this therapeutic is inline dilution and buffer exchanges to remove the ethanol from the formulation and prepare for storage in the final cryo-buffer. While both formulations (LNP1 and LNP2) produced similar CQAs initially (particle size, polydispersity, and EE) at different flow rates and scales (Ignite, Blaze, GMP), following the TFF processing LNP1 showed a significant increase in size, while LNP2 maintained these characteristics. This study demonstrates that some formulations are sensitive to downstream processes, and identifying those CPPs early by testing formulations at a smaller scale can save time, materials, and de-risk the scale-up process.

The NanoAssemblr GMP System has a touchscreen interface, saved recipes, and a single-use fluid path and together with Pall and Cytiva, provides an end-to-end manufacturing workflow for clinical and commercial nanomedicine production. This versatile solution for commercial manufacturing provides the ability to scale up and scale out at a range of facilities.

### 8.7.2 Helix Biotech's Nova™ Benchtop (BT) IJM System

The clinical use of two of the COVID-19 vaccines (BNT162b2 and mRNA-1273) has been a driving force for recognizing the potential of LNPs, which represent an extremely promising technology for delivery of gene products for a host of potential health applications, from immunization against emerging pathogens to personalized neoantigen cancer therapy and gene therapy for many diseases technology for its safety and efficacy. LNPs are usually composed of four lipid types, a cationic/ionizable lipid, a helper phospholipid, a PEGylated lipid, and cholesterol, which together encapsulate and protect nucleic acid cargo. These LNPs are taken up by cells into the endolysosomal system, where a decrease in pH induces the release of the nucleic acid into the cytoplasm where it can exert its effect, either on other RNAs in the case of siRNA cargo, or producing protein, in the case of mRNA or DNA cargos. In order to utilize LNPs most effectively for the variety of potential uses, many different aspects of these tools can be varied to change cell and/or tissue tropism, increase the ability to evade the immune system, or effectively release cargo to the cytoplasm. One of these variable aspects is the cationic/ionizable lipid itself.

The Nova BT platform represents a significant step forward the development of DNA+LNP formulations for gene therapy and gene editing. Overall, observed low particle size (~80 nm), low PDI (~0.2), and high EE (~95%) is observed across all samples with few statistically significant differences. This suggests that Nova BT platform is a robust and reliable tool for small-scale screening and optimization of DNA+LNP formulations. In addition, the platform offers several advantages over existing manufacturing platforms, including greater control over the manufacturing configuration and process via modular plug-and-play design, and better scalability.

The modular platform design allows for easy implementation of additional pumps and mixers for in-line dilution or nanoparticle modification, or increased throughput. The ability to rapidly and efficiently screen, optimize, and scale LNP formulations using our platform will enable researchers to more effectively develop and refine their formulations, potentially leading to more successful clinical outcomes. DNA+LNP nanoparticles have emerged as a promising platform for gene therapy and gene editing with potential applications in a wide range of diseases and conditions. Novel Nova BT platform is highly effective in producing high-quality DNA+LNP nanoparticles, with the ability to optimize formulation and process parameters to achieve desired nanoparticle properties. Our platform offers faster and more efficient production, greater control over the manufacturing process, and better scalability than existing platforms. Their study can contribute to the advancement of gene therapy and gene editing applications using DNA+LNP nanoparticles, and findings will inspire further research in this field (https://www.helixbiotech.com/_files/ugd/1670ed_e5bf3c40d0274c24a27c7594cb46b8a5.pdf accessed on 27 Nov. 2023).

**Helix Biotech's Nova™ Benchtop System** is designed for LNP R&D and preclinical development – screen and optimize nanoparticle formulations quickly and with a versatile and scalable platform. This system utilizes mixing of technologies, Helix Impinged Jet Mixers (IJMs) and Microfluidic (MF) chip.

**Nova™ BT** is a flexible modular platform for LNP synthesis. Researchers can choose from a range of IJM mixers or utilize common and custom microfluidic chips while screening lipid compositions and optimizing drug-loaded formulation process parameters affecting encapsulation, particle size and polydispersity, and stability.

It is a modular system which means that scaling up is as easy as adding modules (pump and/or mixer modules). Simply add a pump module and second mixing stage for in-line dilution or nanoparticle modification capabilities. For larger production capacity, blast through to the Nova Pilot High Throughput (HT) system (https://www.helixbiotech.com/nova?gclid=EAIaIQobChMIk_KykpvNgQMVa6tmAh395grDEAMYASAAEgK3MvD_BwE dated 13 Oct. 2023 21.40 pm).

#### 8.7.2.1 Scale-Up Studies: Moving from ≤1 mL to ≥100 mL

The default Nova™ BT platform including a dual pump module, single-stage mixer, and sample collector can be used to prepare samples as small as 100 uL while also enabling scale-up to ≥100 mL per run. The system provides a repeatable manufacturing process, allowing pooling of multiple batches to produce ≥1 L of material. All Nova™ BT processes and mixing technologies are designed to facilitate seamless tech transfer and process scale-up with SuperNova™ HT systems (Figure 8.9).

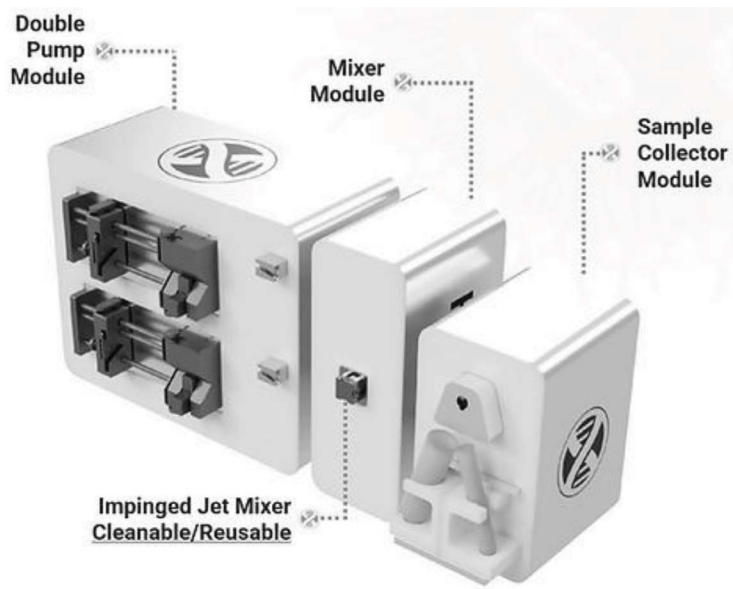



**FIGURE 8.9** Nova™ BT system with dual pump module and single-stage impinged jet mixer (Nova™ IJM: Advanced Lipid Nanoparticle (LNP) manufacturing). (From https://www.helixbiotech.com/nova-lnp-manufacturing-system accessed on 13 Jan. 2025.)

#### 8.7.2.2 Formulation Optimization – Common Easily Tunable Parameters

- TFR – Total flow rate
- FRR – Flow rate ratio
- Process startup/end wastes, priming volumes

#### 8.7.2.3 Application

There are currently dozens of different published lipids, and literature suggests that they can have a significant effect on cargo delivery to different tissues. Helix Biotech examined a common cationic lipid (DOTAP) as well as three well-known ionizable lipids: SM-102, ALC-0315, and DLin-MC3-DMA (MC3). SM-102 and ALC-0315 are the key ionizable lipids in Moderna and Pfizer COVID-19 vaccines, respectively. MC3 is the ionizable lipid in Alnylam's approved siRNA drug, Onpattro® (https://www.helixbiotech.com/_files/ugd/1670ed_acc885d17bfe44cf87afbb496f47f7c2.pdf accessed on 27 Nov. 2023). They have demonstrated production of multiple high-quality LNP formulations and quantitative assays to evaluate the cellular effects of treatment with those LNPs. The hepatocytes were generally the most transfectable cell type for the LNPs tested, which is perhaps unsurprising given that much of literature describes the liver as the primary target of most LNPs. Of the LNP formulations tested, SM-102 was the most efficacious, broadly inducing high levels of GFP expression in all cell types tested. MC3-based particles also had broad efficacy for the cell types tested but did not overall induce as high expression of GFP as SM-102 particles. It is possible that as the ionizable lipid in the siRNA therapy, Onpattro®, MC3 is better at delivering shorter sequences than the longer GFP sequence. The data shown here emphasizes the importance of choosing the best ionizable lipid for the target and payload (https://www.helixbiotech.com/_files/ugd/1670ed_acc885d17bfe44cf87afbb496f47f7c2.pdf accessed on 27 Nov. 2023).

### 8.7.3 The SmartCrystal® Platform of Techniques

It is a combination technology (NanoEdge®, SmartCrystal), commercialized by Abbott labs includes combination technologies such as H42 (spray-drying followed by HPH), H69 (flow precipitation followed by HPH), H96 (lyophilization followed by HPH) (Salazar et al., 2014). There is in-depth research going on for new innovations, for designing numerous different ways to deal with drug NCs. The combination technique involves commonly a pre-treatment step pursued by a high-energy step. For example, the NanoEdge™ innovation. In the pre-treatment step, crystals are precipitated; and resulted suspension is then exposed to high-energy process, like HPH. As described by one of the patent cases of Baxter, the annealing step stops the development of the NCs. Annealing is changing the thermodynamically unstable materials to improve thermodynamically stable structure by single or constant utilization of energy (mechanical pressure or direct heat), followed by thermal relaxation step (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6967137/ accessed on 25 Nov. 2023).

The SmartCrystal® skill was developed and owned by Abbott. It is marketed by its drug delivery company SOLIQS in Ludwigshafen/Germany. SmartCrystal

innovation is not just a single innovation. It has a wide range of procedures that are consolidated to quicken production. It works by reducing the number of passes through the homogenizer to acquire little NCs of size below 100 nm. Such small-sized NCs are not easy to produce through pearl milling or normal HPH, particularly in large-scale mechanical preparation (Raghava Srivalli and Mishra, 2016). The mix procedure H69 is a parallel stream precipitation. It utilizes HPH in which the precipitation is shaped in the cavitation zone or just before the cavitation zone of the homogenizer (caviprecipitation). In the H42 procedure, HPH and spray-drying is mixed. Additionally, in H96 process, the high-weight homogenization (top down) and lyophilization (bottom up) are combined to yield NCs. It is a most effective combination technology. Processes H69 and H96 have the ability to produce crystals below 100 nm, a range practically not accessible by HPH alone. Spray-drying or lyophilization of the drug solution leads to powder, which is more susceptible to get reduced in the subsequent HPH step. The SmartCrystal technology is one of the second generation of drug NCs (Müller and Keck, 2008).

## 8.8 REGULATORY CONSIDERATION OF NANOPARTICLE FORMULATION

### 8.8.1 In India

In India regulation of nanoparticles is still at its initial stage. Through financial assistance, government of India is trying to extend the research of nanotechnology in academic institutions, various public and private R&Ds, and National laboratories. Nanotechnology-based committees were formed in March 2007 by Bureau of Indian Standards (BIS) to consider the standardization for nano devices, sensors, transistors, initiators, etc.

The organization did not include consumer products in their agenda, as standards for these products required advanced technologies and trained personnel (Amenta et al., 2015).

### 8.8.2 In Europe

Nanomedicine developers prepare marketing authorization applications for human medicines for European markets based on the European Medicines Agency's scientific guidelines or Reflection papers on nanomedicines and referring to Scientific guidelines or guidance documents on:

- Data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product.
- Data requirements for intravenous liposomal products developed with reference to an innovator liposomal product.
- Development of block-copolymer-micelle medicinal products.
- Surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products.

### 8.8.3 In the USA

Materials in nanometric sizes can exhibit new or altered physicochemical properties at nanoscale dimensions. This enables the development of novel products. Nanotechnology applications become so exciting because of the various changes in biological, chemical, and other properties. This may have effect on product safety, effectiveness, or other attributes.

The application of nanotechnology may change product attributes and are different from conventionally manufactured products. So, it affects safety or effectiveness of FDA-regulated products that include nanomaterials, which requires thorough evaluation. It involves the application of nanotechnology to consider the unique properties and behaviors that nanomaterials may exhibit. FDA does not categorically consider all nanomaterials and nanotechnology-based products as intrinsically benign or harmful. FDA will regulate nanotechnology-based products under existing statutory authorities, in accordance with the specific legal standards applicable to each type of product under its jurisdiction (https://www.fda.gov/science-research/nanotechnology-programs-fda/fdas-approach-regulation-nanotechnology-products accessed on 25 Nov. 2023).

FDA supports innovation and the safe use of nanotechnology for FDA-regulated products by appropriate and balanced regulatory monitoring. FDA enhances its scientific expertise and tools necessary to assess the safety and effectiveness of products (as applicable). FDA enables the responsible development of products with new and beneficial properties. FDA ensures transparent and predictable regulatory pathways grounded in the best available science. Therefore, FDA's regulatory approach has the following attributes:

- ***FDA is managing by its product-focused, science-based regulatory policy:*** Product-specific technical assessments will be done. It takes into account the impact of nanomaterials on relevant biological and mechanical context of product, and its intended use. So, the particular policies for each product type, will vary according to the statutory authorities in both substantive and procedural manner.
- ***FDA's approach considers variations in legal standards for different product-classes:*** Food additives are considered safe, when there is a reasonable certainty of no harm from their intended use, whereas, drugs, by contrast, are evaluated not only on the basis of their risk profile, but also on the basis of their predicted benefit. These different legal standards show different contexts could lead to different regulatory outcomes, even if two products present the same level of risk. Different other products are subject to yet different standards for safety or effectiveness as regulated by FDA. Even where objective measures of risk are similar, the result is divergent regulatory outcomes for different product classes and different applications of nanomaterials.
- ***Where premarket review authority exists, attention to nanomaterials is given to standing procedures:*** Premarket review requirements is done for new drugs, new animal drugs, biologics, food additives, color additives, certain human devices, and certain new dietary ingredients in dietary

supplements. Premarket review processes for these products need applicants to submit data to answer questions related to the safety, effectiveness (where applicable), or regulatory status of the product. Individual premarket review procedures draw attention toward the use of nanomaterials for additional data on safety or effectiveness, as applicable.

- ***Consultation is promoted to reduce the risk of unintended harm to human or animal health, where statutory authority does not provide for premarket review:*** Premarket review is not mandatory for some FDA-regulated products, such as dietary supplements (except certain new dietary ingredients), cosmetics (except color additives), and food (except food or color additives). For which FDA relies on publicly available or voluntarily submitted information, adverse event reporting (where applicable), and on post-market surveillance activities, to provide oversight. FDA promotes manufacturers to consult agency before taking their products to market, where nanotechnology applications are involved. These consultations help FDA to advise companies, review safety information, and design necessary post-marketing safety oversight.
- ***FDA will continue post-market monitoring:*** In order to protect consumers, FDA monitors the marketplace for products containing nanomaterials and will take actions, as needed.
- ***Industry is responsible for ensuring that its products meet all applicable legal requirements, including safety standards:*** Manufacturers are required to ensure that their product satisfies applicable safety standards and complies with other applicable requirements, regardless of whether products are subject to premarket review or authorization. So, industry has to work with current information in product development, and needs to monitor products once marketed. Industry is expected to consult FDA early, to address questions related to the regulatory status, or to the safety, effectiveness, or other attributes of products that contain nanomaterials or those that involve application of nanotechnology.
- ***FDA will collaborate with domestic and international counterparts on regulatory policy issues:*** FDA is always involved in policy dialogue with other US government agencies, through emerging technologies, interagency policy, coordination committee, and other forums. FDA contributes to overarching US government policies relevant to nanotechnology. FDA will coordinate its policy activities. FDA also works with foreign regulatory counterparts to share perspectives and information for the regulation of nanotechnology products and their intended uses.
- ***FDA will offer technical advice and guidance, as needed, to help industry meet its regulatory and statutory obligations for products that are subjected to premarket review and for those that are not:*** FDA prepares guidance documents for its staff, applicants/sponsors. FDA encourages the public to describe the agency's interpretation of policy on a regulatory issue. Guidance documents emerge over time. Depending upon the product-class, it will address interpretation of relevant statutory and regulatory standards. It provides guidance on technical data needed to meet those standards. FDA will

tailor guidances to the unique confluence of the statute governing the product-class. Further, it is based on the level of scientific knowledge relevant to those applications, and the likely extent of effects on human and animal health.

### Guidance Documents Issued by US-FDA Related to Nanoparticles

1. Final Guidance for Industry – Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients That Are Color Additives
2. Final Guidance for Industry – Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology
3. Final Guidance for Industry – Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation
4. Final Guidance for Industry – Safety of Nanomaterials in Cosmetic Products
5. Final Guidance for Industry – Use of Nanomaterials in Food for Animals
6. Final Guidance for Industry – Drug Products, Including Biological Products, That Contain Nanomaterials

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# 9 Large-Scale Production of Nanocrystals for Pharmaceutical Use

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## 9.1 INTRODUCTION

Nanocrystals have emerged as a promising approach in the pharmaceutical industry to address the challenge of poor water solubility of many drug compounds [1]. Nanocrystals are submicron-sized particles composed of 100% crystalline drug, stabilized by a minimal amount of surfactants or polymeric stabilizers [2]. Nanocrystals are pure drug crystals with sizes in the nanometer range, typically between 10 and 800 nm. They offer several advantages over traditional drug formulations, including high drug loading (up to 100%), improved bioavailability, and ease of scale-up [3]. Marketed pharmaceutical nanocrystal-based products are given in Table 9.1.

Compared to micronized or conventional formulations, nanocrystals exhibit increased dissolution rate due to large surface area, improved saturation solubility, hence enhanced bioavailability and potential for dose reduction [4]. This is achieved through the dramatic increase in surface area-to-volume ratio as the particle size is reduced to the nanometer scale. The increased solubility and dissolution rate can, in turn, lead to improved bioavailability and more consistent pharmacokinetic profiles [5, 6].

In addition to enhancing solubility and bioavailability, the small size of nanocrystals can enable passive targeting to tumors through the enhanced permeability and retention (EPR) effect, while surface functionalization with targeting ligands can further improve tumor accumulation. Several studies have also investigated the use of nanocrystals for cancer treatment. Lü et al. [5, 7] reviewed the development of nanocrystals for cancer therapy, noting that their high drug loading and carrier-free nature make them attractive for delivering anticancer agents.

While laboratory-scale production of nanocrystals is well-established, the pharmaceutical industry faces critical challenges in scaling up these methods to meet commercial demands. This chapter provides a comprehensive analysis of large-scale production techniques, scale-up considerations, process control strategies, and regulatory frameworks. Emphasis is placed on how to maintain product quality, batch-to-batch consistency, and cost-effectiveness while preserving the unique physicochemical attributes that make nanocrystals attractive delivery systems.

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**TABLE 9.1**
**Selected Marketed Pharmaceutical Nanocrystal-Based Formulations**

| Brand Name (Generic Name) | Indication | Company (Initial Developer) | Year of Approval |
|---|---|---|---|
| Rapamune® (Sirolimus) | Immunosuppressant (post-transplant) | Wyeth (Elan) | 2000 (US FDA) |
| Emend® (Aprepitant) | Antiemetic (chemotherapy-induced nausea and vomiting) | Merck (Elan) | 2003 |
| TriCor® (Fenofibrate) | Dyslipidemia (cholesterol/ triglyceride management) | Abbott (Elan) | 2004 |
| Triglide™ (Fenofibrate) | Dyslipidemia | SkyePharma (Elan) | 2005 |
| Gris-PEG® Griseofulvin | Fungal infection | Novartis | 1982 |
| Cesamet® (Nabilone) | Chemo nausea | Lilly | 1985 |
| Triglide™ (Fenofibrate) | Dyslipidemia | Skye Pharma | 2005 |
| EquivaBone® (Hydroxy-apatite) | Bone graft | Zimmer Biomet | 2009 |
| NanOss® (Hydroxy-apatite) | Bone graft | Rti surgical | 2005 |
| OsSatura® (Hydroxy-apatite) | Bone graft | IsoTis Orthobiologics | 2003 |
| Ostim® (Hydroxy-apatite) | Bone graft | Heraseus Kulzer | 2004 |
| Vitoss® (Hydroxy-apatite) | Bone graft | Stryker | 2003 |
| Azopt (Brinzolamide) | Glaucoma | Sandoz | 1998 |
| Megace® ES (Megestrol acetate) | Anorexia | Endo Pharms Inc | 2005 |
| Avinza® (Morphine sulfate) | Chronic pain | Pfizer | 2002 |
| Ritalin LA® (Methylphenidate HCl) | Attention-deficit hyperactivity disorder | Novartis | 2002 |
| Zanaflex® (Tizanidine HCl) | Spasticity | Acorda | 2002 |
| Focalin XR® (Dexmethylphenidate) | Attention-deficit hyperactivity disorder | Novartis | 2005 |
| Invega® (Paliperidone) | Schizophrenia | Janssen Pharmaceuticals | 2006 |
| Invega® Sustenna® (Paliperidone) | Schizophrenia | | 2009 |
| Invega® Trinza® (Paliperidone) | Schizophrenia | | 2015 |
| Invega Hafyera® (Paliperidone) | Schizophrenia | Eagle Pharmaceuticals | 2014 |
| Ryanodex® (Dantrolene Sodium) | Malignant hyperthermia | Alkermes | 2015 |
| Aristada® (Aripiprazole Lauroxil) | Schizophrenia | Sun Pharma | 2018 |
| Yonza® (Abiraterone Acetate) | Prostate cancer | Baudax Bio | 2020 |
| Anjeso® (Meloxicam) | Pain | ViiV Healthcare | 2021 |
| Vocabria (Cabotegravir) | HIV | Janssen Pharma/ ViiV Healthcare | 2021 |

## 9.2 NANOCRYSTAL FORMATION MECHANISMS

A comprehensive understanding of nanocrystal formation mechanisms is indispensable for rationally designing materials with tailored properties. Over the past decades, numerous studies have delineated the key steps of nucleation, growth, and Ostwald ripening, as well as more complex pathways involving intermediate phases, precursor decomposition, and template-guided assembly [8–10].

### 9.2.1 Classical Nucleation and Growth Mechanisms

Early theoretical frameworks for nanocrystal formation are often rooted in classical nucleation theory (CNT). Within CNT, the initial formation of atomic or molecular clusters in solution is viewed as a stochastic event, resulting from random fluctuations in local concentration and thermal energy. Once a stable cluster form exceeds a critical size at which the system's free energy is minimized, growth occurs predominantly by the addition of monomers to the nascent crystal [8]. As growth proceeds, the particle size distribution narrows under conditions that favor monomer depletion. This classical picture remains an invaluable reference, but actual systems frequently exhibit deviations that can be attributed to non-classical pathways or the influence of impurities, surfactants, and complex reaction chemistries [11].

In practice, solution-based colloidal syntheses often rely on the rapid injection of precursors to achieve supersaturation, followed by a nucleation "burst" that reduces the monomer concentration. Subsequent growth steps occur under conditions of diminished supersaturation, leading to uniform nanocrystal sizes. Hence, temporal separation of nucleation and growth stages is a crucial parameter for achieving monodisperse nanoparticles [12]. The addition of surfactants, ligands, and capping molecules is a strategic approach to modulate growth kinetics and stabilize crystal facets, ultimately directing the morphology and size distribution of the final nanocrystals [13].

#### 9.2.1.1 Non-Classical Pathways and Intermediate Phases

Although CNT provides a conceptual baseline, non-classical nucleation pathways are now widely recognized. Such mechanisms often involve the formation of amorphous precursors, clusters, or metastable intermediate phases that gradually reorganize into crystalline structures through oriented attachment or other self-assembly processes [10, 14]. For instance, in the formation of metal oxide nanocrystals, colloidal clusters may initially lack long-range order, only to crystallize upon further heating or through interaction with stabilizing ligands.

Oriented attachment—where discrete nanocrystals spontaneously align and fuse along specific crystallographic orientations—represents a notable non-classical growth mechanism. This process reduces the total surface energy and leads to anisotropic growth and the development of hierarchical architectures that cannot be easily explained by monomer-by-monomer addition [14].

### 9.2.2 Ostwald Ripening and Size Focusing Mechanisms

Following nucleation and initial growth, nanocrystal populations typically undergo size redistribution processes. Ostwald ripening, a phenomenon wherein larger particles grow at the expense of smaller ones, plays a crucial role in defining the final size distribution [11]. The thermodynamic driving force for Ostwald ripening is the reduction of the system's overall surface free energy, leading to a shift in the size distribution toward fewer, larger crystals over time.

Counterbalancing or controlling Ostwald ripening is key to achieving the narrow size distributions desired for many applications. Strategies to limit ripening include the use of ligands that selectively bind to particular facets, the continuous

replenishment of monomers, or the introduction of minor components that stabilize smaller particles. In some systems, "size-focusing" regimes can be induced, wherein nanocrystal sizes converge over time to a narrow distribution, guided by careful tuning of reaction parameters and precursor chemistry [9, 13].

The precursors and solvents employed in a nanocrystal synthesis profoundly impact nucleation and growth. Thermal decomposition methods, for example, rely on the slow and controlled release of monomers from organometallic complexes at elevated temperatures. The nature of these complexes and their decomposition pathways can drastically affect the nucleation burst, growth rates, and resultant nanocrystal morphology [9]. Similarly, the choice of solvent not only dictates the solubility and diffusion of monomers but also governs ligand coordination chemistry, surface energies, and colloidal stability [15].

Template-directed synthesis presents another dimension to nanocrystal formation. Porous membranes, surfactant micelles, and biological scaffolds can act as confined reaction environments or structurally guiding frameworks, encouraging the formation of particular shapes, composites, or assemblies of nanocrystals [16]. By controlling local chemical potentials and steric constraints, such templates yield complex architectures or hierarchical materials that would be difficult to achieve under bulk conditions.

## 9.3 APPROACHES TO NANOCRYSTAL PRODUCTION

There are two main approaches for the preparation of drug nanocrystals: top-down and bottom-up techniques [17]. While top-down techniques, such as high-pressure homogenization (HPH) and media milling, have been successfully used at the commercial scale, bottom-up techniques have not yet been widely adopted for industrial-scale production [17, 18].

### 9.3.1 Top-Down Methods

Top-down approaches involve the mechanical reduction of larger drug crystals into nanoparticles. They are often favored at industrial scales due to their direct scalability.

#### 9.3.1.1 Wet Milling

Wet milling is a widely used top-down technique for the production of drug nanocrystals [19, 20]. This method utilizes ball milling with milling media (glass, zirconium, or stainless-steel balls) to reduce the particle size of the drug to the nanometer range [21]. The wet milling process can lead to a slight reduction in the crystallinity or partial amorphization of the drug, which can explain the higher solubility of the resulting nanocrystals compared to the unprocessed powder [22].

Careful selection of appropriate stabilizers is crucial for the formation of stable nanosuspensions [21]. Commonly used stabilizers include polymers, celluloses, surfactants, and lipids [23]. The stabilizers help prevent particle agglomeration and Ostwald ripening, thereby improving the physical stability of the nanocrystals [24]. The drug-to-stabilizer ratio is an important parameter, as lower stabilizer concentrations may not be sufficient to form stable nanocrystals [25].

Wet milling has significant potential for the large-scale production of commercially viable nanocrystal products due to its ease of scale-up [21]. Several nanocrystal products approved by the US Food and Drug Administration (FDA) have been prepared using wet ball milling techniques [26]. The small domain size of the nanocrystals (typically 100–200 nm) can result in only a slight reduction in the desired properties compared to the glassy matrix [27].

In addition to improving the solubility and bioavailability of poorly soluble drugs, nanocrystals have also been explored for various other pharmaceutical applications, such as transdermal delivery [28], targeted drug delivery [29], and parenteral administration [1]. The combination of nanocrystal techniques with other approaches, such as co-crystallization, has also been investigated to further enhance the properties of poorly soluble drugs [30].

Overall, the wet milling method is a well-established and scalable technique for the large-scale production of drug nanocrystals, which can significantly improve the physicochemical and biopharmaceutical properties of poorly soluble drugs [31, 32].

#### 9.3.1.2 High-Pressure Homogenization

HPH forces a drug suspension through a narrow gap at pressures up to 4000 bar, resulting in particle cavitation and collision-induced fracture [33]. Scalable systems with automated controls allow continuous processing and straightforward scale-up. HPH is a widely used technique in the pharmaceutical industry for the production of nanocrystals [34].

The HPH process involves subjecting a suspension of the drug and stabilizers to high pressures, typically ranging from 100 to 400 MPa, which causes the drug particles to break down to nanoscale size through mechanisms such as cavitation, shear forces, and collision [35]. Additionally, in-line nanoparticle size measurements could be performed using the NanoFlowSizer (NFS) in HPH to achieve real-time process control [36].

Several studies have demonstrated the effectiveness of HPH in producing nanocrystals of various poorly soluble drugs, such as curcumin [37], quercetin [38, 39], and ZGDHu-1 [40]. The optimized HPH conditions, including temperature, pressure, and number of cycles, have been reported to achieve the desired particle size, crystallinity, and stability of the nanocrystals [37, 40].

In addition to particle size reduction, HPH can also influence other physicochemical properties of the nanocrystals, such as zeta potential, dispersity, and viscosity [37]. The high shear forces and pressure during HPH can potentially cause structural changes in the stabilizing agents, such as chitosan, which may affect the zeta potential and stability of the nanoparticles [41].

The advantages of using HPH for the production of nanocrystals include its simplicity, efficiency, and environmental friendliness, as it does not require the use of organic solvents. Furthermore, HPH has been widely used in the food, cosmetic, and pharmaceutical industries for various applications, such as the preparation of emulsions, disruption of microorganisms, and modification of biopolymer structures. However, it is important to note that the application of very high pressures during HPH can potentially lead to the damaging of long-chain molecules, such as proteins and polysaccharides, which may affect their functional properties [42]. Therefore,

the optimization of HPH conditions is crucial to achieve the desired physicochemical and functional properties of the nanocrystals.

### 9.3.2 Bottom-Up Methods

Bottom-up approaches rely on controlled precipitation from solutions. Although they often providing uniform particle sizes and stable polymorphs, they are more challenging to scale due to the sensitivity of nucleation and growth kinetics. Bottom-up techniques, also known as precipitation techniques, involve the self-assembly of drug molecules into nanocrystals [43]. These techniques include antisolvent precipitation, controlled solvent evaporation, antisolvent precipitation under ultrasound or supercritical fluid, spray-drying, and freeze-drying [44].

Antisolvent precipitation is a bottom-up technique where the drug is dissolved in a solvent and then precipitated by the addition of an antisolvent [45]. A controlled precipitation occurs when drug solubility changes abruptly by adding an antisolvent. Controlled solvent evaporation involves slowly evaporating the solvent from a drug solution, leading to the formation of nanocrystals [45]. These techniques are relatively simple but may result in broader particle size distributions [45]. Scale-up demands precise control of solvent ratios, flow rates, and temperature to maintain consistent particle size distribution. Achieving uniform mixing on a large scale requires advanced mixing technologies, such as static mixers or impinging jet reactors [46].

Antisolvent precipitation under ultrasound or supercritical fluid can also be used to produce nanocrystals [45]. Ultrasound can enhance the nucleation and growth processes, leading to smaller and more uniform nanocrystals. Supercritical fluids, such as carbon dioxide, can act as antisolvents and facilitate the precipitation of nanocrystals [45].

Spray-drying and freeze-drying are two commonly used techniques for the large-scale production of drug nanocrystals [45, 47, 48]. Spray-drying involves the atomization of a drug solution or suspension into fine droplets, which are then rapidly dried, resulting in the formation of nanocrystals [45, 48]. This method is scalable and can produce nanocrystals with controlled size and morphology. Freeze-drying, on the other hand, involves the freezing of a drug solution or suspension, followed by the sublimation of the solvent under vacuum [47, 49]. Freeze-drying can preserve the nanocrystal structure and prevent Ostwald ripening during the drying process.

The choice of drying method can significantly impact the properties of the resulting nanocrystals. For example, the lack of freezing in spray-drying can minimize oil droplet agglomeration, making it a faster and more scalable technique compared to freeze-drying [50]. However, freeze-drying may be more suitable for thermally labile drugs, as it can preserve the nanocrystal structure better [47, 49]. The use of cryoprotectants, such as mannitol or trehalose, can also improve the re-dispersibility of freeze-dried nanocrystals [51].

In addition to the drying method, other factors, such as the type and concentration of stabilizers, can also influence the properties and stability of the resulting nanocrystals [52]. The choice of stabilizers, such as hydroxypropyl methylcellulose, hydroxypropyl cellulose, or poloxamers, can affect the dissolution performance and long-term stability of the nanocrystals [52].

Overall, the selection of the appropriate precipitation and drying technique for the large-scale production of drug nanocrystals depends on the specific properties of the drug, the desired nanocrystal characteristics, and the scalability and efficiency of the process [45, 47].

Compared to top-down methods, bottom-up approaches have the potential to produce nanocrystals with superior crystallinity, narrow particle size distribution, and uniform morphology, while requiring less energy and causing fewer issues with contamination from manufacturing equipment [7, 45]. One of the key advantages of bottom-up techniques is their ability to lock the intramolecular motions of organic molecules, which can help to block the vibrational relaxation pathways and enhance the performance of the nanocrystals [53]. Additionally, bottom-up approaches, such as the solvent exchange method (also known as the reprecipitation method), do not require excess energy to form the organic nanocrystals, making them suitable for large-scale production [54]. Despite these advantages, the scale-up of bottom-up techniques for industrial-scale production remains a challenge [55]. Researchers have explored various strategies to address this issue, such as the use of porous hollow fiber-based antisolvent crystallization (PHFAC) modules for continuous production of nanocrystals [56].

One promising approach for the large-scale production of nanocrystals is continuous flow synthesis. Compared to batch production, continuous flow reactors have shown great potential for the scalable and controlled synthesis of colloidal nanocrystals [57]. For example, Shavel et al. developed a procedure for the continuous production of $Cu_2ZnSnS_4$ (CZTS) nanoparticles using a flow reactor, where the reaction of metal amino complexes with elemental sulfur at 300–330°C produced nanocrystals with controlled composition [58]. Similarly, Yarema et al. reported an approach to upscale hot-injection syntheses of various nanocrystals (Sn, PbS, $CsPbBr_3$, $Cu3In_5Se_9$) by 1–2 orders of magnitude using a reactor with mild underpressure to facilitate rapid precursor addition [59].

The continuous flow approach offers several advantages over batch synthesis. Niu et al. highlighted that droplet reactors, in particular, have shown great potential for the continuous and scalable production of colloidal nanocrystals with uniform and well-controlled properties [57]. The authors noted that this approach can help bridge the gap between academic studies and industrial applications by enabling large-scale nanocrystal production without compromising quality.

### 9.3.3 Combined and Hybrid Methods

Combining top-down and bottom-up principles can offer better control over particle size distribution and stability. Hybrid processes like nano-precipitation followed by mild homogenization can potentially streamline large-scale manufacturing. Top-down methods, such as HPH and media milling, involve the mechanical size reduction of larger drug particles to the nanoscale [1, 16, 60]. These techniques have been successfully used in both laboratory- and commercial-scale manufacturing [16]. On the other hand, bottom-up approaches, such as antisolvent precipitation, involve the controlled crystallization of drug molecules from solution to form nanocrystals [1, 60]. While bottom-up methods have not yet been widely adopted at a commercial level, they have been found to produce nanocrystals with a narrow size distribution using simple methods [16].

Top-down methods involve the mechanical size reduction of larger drug particles to the nanoscale, typically using techniques such as wet milling or HPH [5, 61]. These approaches are scalable and applicable to a wide range of drugs with different physicochemical and mechanical properties [62, 63]. However, they may require repeated processing steps and can potentially introduce impurities or structural changes to the drug [64, 65].

Bottom-up methods, on the other hand, involve the controlled precipitation or self-assembly of drug molecules to form nanocrystals [66, 67]. These techniques, such as antisolvent precipitation, can produce nanocrystals with a high drug loading capacity and minimal use of excipients [68, 69]. However, they may face challenges in terms of scalability and the need for precise control over the crystallization process [70].

To leverage the advantages of both top-down and bottom-up approaches, researchers have explored the use of combined and hybrid methods for the large-scale production of nanocrystals [45]. These combined methods merge the favorable features of each process and avoid the disadvantages of single processes. For example, the porous hollow fiber-based antisolvent crystallization (PHFAC) module, which combines top-down and bottom-up principles, has been successfully used to fabricate nanocrystals under moderate conditions and has been scaled up for continuous production [71].

One example of a hybrid method is the combination of wet milling and antisolvent precipitation, where the initial bottom-up precipitation step is followed by top-down milling to further reduce the particle size and improve the physicochemical properties of the nanocrystals [72]. This approach can result in nanocrystals with enhanced dissolution rates, improved bioavailability, and better stability compared to those produced by either method alone [73]. Another combined approach involves the use of top-down techniques, such as high-pressure homogenization, in conjunction with bottom-up strategies, such as the addition of stabilizers or surface modifiers [74]. This combination can help to control the particle size, morphology, and surface characteristics of the nanocrystals, leading to improved targeting, cellular uptake, and drug delivery efficiency.

Another example of a combined approach is the use of droplet reactors for the synthesis of colloidal nanocrystals [57, 75]. Droplet reactors offer the potential for high-throughput production of nanocrystals by combining the advantages of both homogeneous nucleation and seed-mediated growth. The use of an inert-gas-driven microflow reactor has been reported as a viable and robust strategy for the continuous production of high-quality colloidal nanocrystals [76].

Additionally, the combination of top-down and bottom-up techniques has been explored for the production of drug nanocrystals and nano co-crystals. This approach, known as "confinement methods," has been found to be applicable at both the engineering and pharmaceutical levels [45].

## 9.4 SCALE-UP CONSIDERATIONS

The scale-up of nanocrystal manufacturing for pharmaceutical applications faces several challenges, including nanocrystals stability and dispersity [77, 78], environmental considerations, [79, 80] and process optimization and automation of the

**TABLE 9.2**
**Key Parameters Affecting Large-Scale Nanocrystal Production**

| Parameter | Influence on Nanocrystals | Scale-Up Challenges |
|---|---|---|
| Milling speed/pressure | Particle size reduction | Maintaining uniform energy input |
| Stabilizer concentration | Particle stability | Cost and regulatory limitations |
| Solvent/antisolvent ratio | Particle size distribution | Ensuring consistent mixing |
| Temperature control | Polymorphic form stability | Large-scale heat exchange |
| Residence time (continuous) | Particle growth and size | Managing flow rates at large scale |

nanocrystal manufacturing process are necessary to achieve consistent quality and high yield at a large scale in an environmental friendly approach [81, 82]. The key scale-up challenges of different methods are depicted in Table 9.2.

The production of nanocrystals for pharmaceutical applications requires careful consideration of scale-up factors to ensure consistent quality and yield. One crucial aspect is the precise control of stoichiometry during the synthesis process, as it directly impacts the passivation and properties of the nanocrystals [83]. Achieving true nucleation control in aggregative growth is essential for size control and reproducibility in nanocrystal synthesis [84].

The selection and optimization of equipment for the large-scale production of nanocrystals are crucial to ensure consistent quality and high yield. High-pressure homogenizers, industrial bead mills, and continuous-flow precipitation systems must be engineered to handle large volumes and maintain consistent shear forces and energy inputs. Efficient mixing and dispersion are essential for achieving uniform nanocrystal size and morphology. The parameters that influence particle size and distribution include rotation speed (for milling), homogenization pressure, temperature, solvent-to-antisolvent ratio, and stabilizer concentration. Implementing process analytical technology (PAT) tools and real-time monitoring facilitates tighter control over these parameters.

Top-down approaches, such as wet stirred media milling (WSMM), are considered more promising for large-scale production of drug nanoparticles compared to bottom-up methods [85]. WSMM can achieve high drug loading, organic solvent-free processing, continuous operation, and ease of scale-up. Other top-down techniques, like HPH and wet bead milling, have also been successfully employed to formulate poorly soluble drugs at the commercial scale [86].

In contrast, bottom-up approaches, such as precipitation, can suffer from limitations like low drug loading, presence of organic solvents, and thermodynamically unstable particles [85]. However, bottom-up methods can still be optimized for large-scale production through the use of design of experiments (DoE) techniques. DoE has been applied to investigate the correlation between energy input, drug-related, and stabilizer-related aspects for both top-down and bottom-up nanocrystal production [87].

The heat-up (non-injection) method for the synthesis of colloidal nanocrystals has been identified as a reliable and scalable approach, as it can produce high-quality

nanomaterials with little or no batch-to-batch variation [88]. This method involves heating the precursors within a reaction medium to induce a chemical reaction that yields monomers for nucleation and growth.

Careful control of reaction kinetics and thermodynamics is crucial for the formation of monodisperse nanocrystals, as the kinetics of the chemical reaction can significantly impact the size and size distribution of the nanocrystal ensemble [89]. Novel synthetic approaches, such as the use of thermodynamic equilibrium-driven formation, have been explored to achieve single-sized nanocrystals [89].

The drying of drug-laden nanosuspensions to form nanocomposites is a critical step in the scale-up process. Techniques like spray drying and hot-melt extrusion have been explored to achieve this in a continuous and scalable manner [90, 91]. Stabilizers prevent particle agglomeration and Ostwald ripening. Common stabilizers include poloxamers, polyvinylpyrrolidone (PVP), and lecithin [60]. On a large scale, the cost, regulatory status, and compatibility of these stabilizers must be considered.

Continuous flow reactors, such as microfluidic devices or centrifugal flow reactors, have been shown to provide excellent control over mixing and dispersion [92, 93]. Precise control over temperature and pressure is necessary to maintain the desired supersaturation and reaction kinetics during nanocrystal formation. Continuous flow reactors and hydrothermal synthesis approaches can provide excellent control over these parameters [94, 95]. Continuous processing offers advantages in scale, reproducibility, and cost reduction. However, the transition from batch to continuous operation requires robust process control and validation to ensure uniform product quality. In-line monitoring and control of critical process parameters, such as particle size, morphology, and crystallinity, are essential to ensure consistent product quality during scale-up. Techniques like optical coherence tomography and X-ray diffraction (XRD) have been used for this purpose [96, 97].

In addition to the synthesis method, the downstream processing and formulation of nanocrystals are also crucial for their pharmaceutical applications. Malamatari et al. [3] reviewed various preparation methods and downstream technologies for developing tailor-made nanocrystal-based drug delivery systems. The authors highlighted that nanocrystals can be used to improve the solubility and bioavailability of poorly soluble drugs, and can be administered through various routes, including oral, parenteral, pulmonary, ocular, and dermal. Additionally, the use of automated platforms for the reproducible synthesis and high-throughput optimization of colloidal nanocrystals has been reported [98]. These robotic platforms enable precise control over reaction conditions and can perform workflows analogous to traditional flask syntheses, facilitating the optimization of physical properties relevant to emerging applications.

## 9.5 CHARACTERIZATIONS OF NANOCRYSTALS

Nanocrystals are characterized by their small particle size, typically ranging from 10 to 800 nm [2]. This small size results in a large surface area-to-volume ratio, which is a key property that enhances the dissolution rate and bioavailability of poorly soluble drugs [1, 99]. The choice of characterization tools and methods depends on

the specific properties of the nanocrystals and the information required for pharmaceutical development and optimization.

Several characterization techniques are employed to analyze the properties of pharmaceutical nanocrystals. High-resolution transmission electron microscopy is used to study the atomic-scale structure and morphology of nanocrystals [100]. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) provide information on the size, shape, and surface characteristics of nanocrystals [2]. Dynamic light scattering (DLS) is a common technique used to measure the hydrodynamic size distribution of nanocrystals in suspension [2].

XRD is used to analyze the crystalline structure and polymorphic form of nanocrystals [2]. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) provide information on the thermal behavior and phase transitions of nanocrystals. Zeta potential measurements are used to evaluate the surface charge and stability of nanocrystal suspensions [2]. Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) can provide insights into the surface chemistry and adsorption of stabilizers on nanocrystals.

Dialysis techniques are commonly used to study the release kinetics of drug nanocrystals, although the results may not fully represent the *in vivo* behavior. Physiologically based pharmacokinetic (PBPK) modeling can be employed to elucidate the *in vivo* fate and disposition of drug nanocrystals [101].

## 9.6 CRITICAL QUALITY ATTRIBUTES FOR NANOCRYSTALS

The critical quality attributes (CQAs) for nanocrystals are the physical, chemical, and biological properties that must be within an appropriate range to ensure the desired product performance and quality [102, 103]. Thorough validation of the nanocrystallization process, including parameters like milling time, temperature, and energy input, is necessary to ensure consistent product quality and reproducibility [104].

Comprehensive analytical techniques, such as DLE, XRD, and electron microscopy, are employed to thoroughly characterize the nanocrystal properties, including size, morphology, crystallinity, and drug content. The average particle size and narrow size distribution are critical to achieve the desired dissolution rate and bioavailability [1, 16, 105–109]. Maintaining the desired crystalline form and preventing unwanted polymorphic transitions is essential for the stability and performance of the nanocrystal formulation [110].

Accurate quantification of the drug content and ensuring the absence of impurities and degradation products are crucial for the safety and efficacy of the nanocrystal-based drug product [111]. The ability of the nanocrystals to be easily redispersed without aggregation and maintain their physical stability during storage and handling is a critical attribute [103, 112]. The choice and concentration of stabilizers, such as surfactants and polymers, play a crucial role in preventing particle aggregation and maintaining the physical and chemical stability of the nanocrystals. Lastly, the dissolution rate and drug release characteristics of the nanocrystals must be thoroughly evaluated to ensure the desired pharmacokinetic and pharmacodynamic properties [1, 16, 105–109].

## 9.7 REGULATORY CONSIDERATIONS FOR NANOCRYSTALS

Nanocrystals have emerged as a promising approach for enhancing the solubility and bioavailability of poorly water-soluble drugs. However, their unique physicochemical properties pose distinct regulatory challenges [113]. The size, morphology, and surface characteristics of nanocrystals can significantly influence their pharmacokinetics, biodistribution, and potential toxicity, necessitating comprehensive evaluation during development. Regulatory agencies, including the US FDA and the European Medicines Agency (EMA), emphasize the importance of rigorous characterization and quality control for nanocrystalline formulations. Parameters such as particle size distribution, zeta potential, polymorphic stability, and dissolution profile are critical to ensuring batch-to-batch consistency and product safety. Additionally, the potential for aggregation, altered biodistribution, and unexpected interactions with biological systems requires detailed *in vitro* and *in vivo* studies to assess safety and efficacy.

Another significant consideration is the classification of nanocrystals as either novel or established entities under regulatory frameworks, which may vary depending on their composition and intended use. For instance, nanocrystals comprising existing active pharmaceutical ingredients (APIs) may follow an abbreviated regulatory pathway, such as the 505(b)(2) pathway under the FDA, if there is sufficient evidence of similarity to previously approved products [114]. Conversely, novel APIs formulated as nanocrystals often require full clinical evaluation due to potential differences in pharmacodynamic and toxicological profiles. Furthermore, the regulatory landscape for nanocrystals is evolving, with guidelines such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q8-Q10 providing a structured framework for Quality by Design (QbD) approaches in their development [115]. These approaches are particularly critical for nanocrystals, where subtle changes in manufacturing processes can significantly affect the final product's performance.

Given the interdisciplinary nature of nanocrystal development, collaboration between pharmaceutical scientists, toxicologists, and regulatory experts is essential to navigate these complexities effectively. The integration of emerging tools, such as advanced analytical techniques and predictive modeling, can further streamline the regulatory approval process while ensuring patient safety. However, challenges remain, including harmonizing global regulatory standards and addressing the lack of specific guidelines tailored to nanomedicine. Future advancements in regulatory science and ongoing dialogue between stakeholders are crucial for the successful translation of nanocrystals from bench to bedside.

## 9.8 CONCLUSIONS

The large-scale production of nanocrystals is a mature yet evolving field. The scale-up of nanocrystal production for pharmaceutical use requires a multifaceted approach that considers factors such as stoichiometry control, nucleation kinetics, equipment selection (top-down vs. bottom-up), and process optimization techniques like DoE and automated platforms. Advances in continuous manufacturing technologies, PAT, and predictive modeling tools are expected to further streamline

scale-up efforts. As more complex molecules enter the pipeline, innovation in both top-down and bottom-up methodologies will be needed to maintain product quality and cost-effectiveness.

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# 10 Large-Scale Production of Inorganic/Metallic Nanoparticles

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## 10.1 INTRODUCTION

Inorganic and metallic nanoparticles have garnered significant attention in the pharmaceutical field due to their unique properties and potential applications [1]. Figure 10.1 depicts different metallic and inorganic nanoparticles that are used in pharmaceutical applications. These nanoparticles can be engineered to serve as effective drug delivery systems (DDS), imaging agents, and therapeutic platforms. Table 10.1 shows details of marketed products based on these nanoparticles.

Some products containing metallic or inorganic nanoparticles are approved as medical devices or contrast agents rather than traditional pharmaceuticals. Quantum

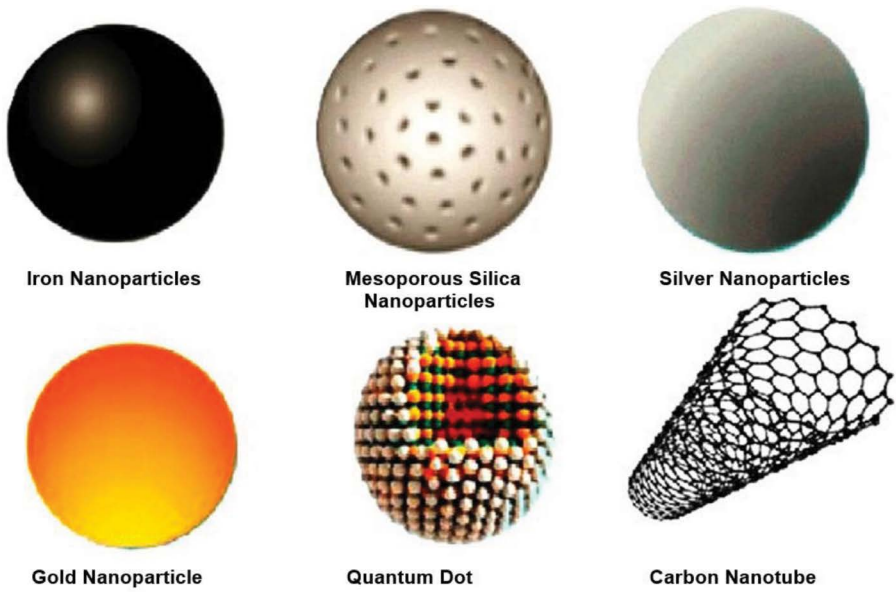


**FIGURE 10.1** Types of metallic/inorganic nanoparticles.


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**TABLE 10.1**
**Marketed Products Based on Inorganic/Metallic Nanoparticles**

| Category | Product Name/ Trade Name | Manufacturer/ Developer | Nanomaterial Composition | Primary Indication/Use | Regulatory Status/Notes |
|---|---|---|---|---|---|
| Inorganic/ Metallic NPs | Feraheme (Ferumoxytol) | AMAG Pharmaceuticals | Iron oxide nanoparticles | Treatment of iron-deficiency anemia in CKD | FDA approved (US) |
| | Feridex IV (Ferumoxides) | Advanced Magnetics (acquired by AMAG) | Iron oxide nanoparticles | MRI contrast agent (liver imaging) | FDA approved in 1996; discontinued in 2009 |
| | Resovist (Ferucarbotran) | Bayer Schering Pharma | Iron oxide nanoparticles | MRI contrast agent (liver imaging) | Approved in EU and Asia; not in US |
| | Hensify (NBTXR3) | Nanobiotix | Hafnium oxide nanoparticles | Radioenhancer for soft tissue sarcoma therapy | CE marked in EU; in clinical trials in US |
| | Acticoat (Wound Dressing) | Smith & Nephew | Silver nanoparticles | Antimicrobial wound dressing | Approved as a medical device (wound care) |
| | AuroLase Therapy (using AuroShell® particles) | Nanospectra Biosciences | Gold nanoshells | Photothermal ablation of solid tumors (e.g. prostate cancer) | In clinical trials; not yet FDA-approved for broad market use |
| Quantum Dots | - | - | Semiconductor nanocrystals (e.g., CdSe/ZnS) | Potential imaging/ diagnostic agents under research | Primarily in R&D and clinical research; no mainstream regulatory approvals |
| Carbon-based Nanomaterials | - | - | Fullerenes, carbon nanotubes, graphene derivatives | Potential drug delivery, imaging, antioxidant, and therapeutic carriers (in research) | Primarily in preclinical or clinical research; no widely approved products |

dots (QDs) and carbon-based nanomaterials are under intense investigation for pharmaceutical applications, but as of now have not achieved mainstream regulatory approval in marketed therapeutic products.

Metallic nanoparticles have been explored for a wide range of biomedical applications, such as cancer diagnosis, thermal ablation, radiotherapy, and the management of neurological disorders [2]. Their unique optical, magnetic, and chemical properties can be leveraged for various imaging and therapeutic modalities, including magnetic resonance imaging (MRI), photothermal therapy, and particle-induced radiation therapy [3]. These nanoparticles, including gold, silver, and magnetic nanoparticles, possess a high surface area-to-volume ratio, stability, and versatile surface chemistry [4]. The biocompatibility and multifunctionality of metallic nanoparticles make them ideal drug delivery vehicles [4]. They can be conjugated with ligands, imaging labels, therapeutic drugs, and other functional moieties to enable targeted drug delivery and enhance bioavailability [5, 6]. Additionally, the stability and biocompatibility of metal nanoparticle-based DDS have been extensively studied, highlighting their potential for clinical applications [7].

Furthermore, the surface modification of metallic nanoparticles can enhance their biocompatibility and targeting capabilities, rendering them more effective as DDS [7]. For instance, gold, silver, and platinum nanoparticles have been extensively studied for their potential in cancer therapy, either as drug delivery vehicles or as therapeutic agents themselves [8, 9]. The encapsulation of drugs within polysaccharide-loaded metal nanoparticles has been investigated as a strategy to overcome challenges such as limited drug delivery, active cellular efflux, and inadequate carrier systems [10].

Inorganic nanoparticles have emerged as promising candidates for various biomedical applications, including drug delivery, imaging, and therapeutic interventions [11, 12]. These nanomaterials possess unique physicochemical properties, such as size-dependent optical, electrical, and magnetic characteristics, which make them attractive for use in the pharmaceutical field [11, 13, 14].

Inorganic nanoparticles are typically formed through the crystallization of inorganic salts, resulting in a three-dimensional arrangement of linked atoms [11]. The ability to tailor the size, shape, and surface properties of these nanoparticles allows for the development of targeted DDS and the enhancement of therapeutic efficacy [8, 12]. The unique properties of inorganic nanoparticles, such as superparamagnetism, upconversion, and size-dependent absorbance/emission, have enabled their use in various biomedical applications, including diagnostic imaging, hyperthermia, and theranostic approaches [12, 15, 16]. The surface functionalization of these nanoparticles with polymers or biological membranes can also improve their biocompatibility, circulation time, and tumor-targeting capabilities, thereby enhancing their therapeutic efficacy [17, 18].

Despite the promising potential of inorganic nanoparticles, there are still challenges that need to be addressed, such as ensuring long-term *in vivo* biocompatibility, overcoming systemic toxicity, and improving biodegradability [19, 20]. Ongoing research is focused on developing strategies to address these issues, such as the use of hybrid nanoparticle systems that combine inorganic

and organic components [21–23]. One class of inorganic nanoparticles that has shown promise for pharmaceutical applications is layered double hydroxides (LDHs) [24]. LDH nanoparticles can be functionalized with cationic polymers, such as poly(DMAEMA), to enhance their DNA-condensing ability and improve their biocompatibility for gene delivery applications [25]. Additionally, LDH nanoparticles have been used to co-deliver small interfering RNA (siRNA) and anti-cancer drugs, demonstrating their potential as multifunctional drug delivery carriers [26]. Another type of inorganic nanoparticles that has been explored for pharmaceutical use are mesoporous silica nanoparticles (MSNs). MSNs have been proposed as ideal drug delivery carriers due to their high surface area, tunable pore size, and ability to co-deliver siRNA and anti-cancer drugs, such as doxorubicin [27, 28].

Carbon-based nanomaterials, including carbon nanotubes (CNTs), have also garnered significant attention in the pharmaceutical field. CNTs possess exceptional physicochemical properties, such as high surface area, high aspect ratio, and the ability to cross biological barriers, making them promising candidates for drug delivery and biomedical imaging [29]. However, the potential toxicity of CNTs remains a concern, and extensive biocompatibility studies are required before their widespread adoption in pharmaceutical applications.

QDs, a class of semiconductor nanocrystals, have also been explored for biomedical applications due to their unique optical and electronic properties [29]. These nanomaterials exhibit discrete energy levels and can be used for bioimaging, biosensing, and theranostic applications [30]. However, the potential toxicity of QDs, particularly those containing heavy metals like cadmium, remains a concern, and the development of biocompatible QDs, such as silicon-based QDs, is an active area of research [31].

In addition to the aforementioned inorganic nanoparticles, other types of hybrid nanoparticles, such as lipid-coated calcium phosphate (LCP) nanoparticles [32] and polyethyleneimine (PEI)-coated amorphous calcium carbonate (ACC) nanoparticles [33], have also been explored for pharmaceutical applications. These hybrid nanoparticles can combine the advantages of both organic and inorganic components, offering improved stability, biocompatibility, and drug/gene delivery capabilities.

Table 10.2 summarizing various types of inorganic, metallic, carbon-based, and QD nanomaterials, as well as selected specialized nanosystems (such as LDHs, PEI-ACC, and LCP nanoparticles) for pharmaceutical applications, including their key properties and main uses.

Overall, inorganic and metallic nanoparticles, including LDHs, MSNs, CNTs, QDs, and hybrid nanoparticles, have shown great potential for pharmaceutical applications, such as drug delivery, gene delivery, bioimaging, and biosensing. However, the potential toxicity of some of these nanoparticles, particularly those containing heavy metals, remains a concern, and the development of biocompatible alternatives is an active area of research. Continued advancements in the synthesis, functionalization, and characterization of these nanoparticles will be crucial for their successful translation into clinical applications. Here is a detailed discussion of the different methods used for the large-scale production of inorganic and metallic nanoparticles, CNTs, and QDs for pharmaceutical use:

**TABLE 10.2**
**Different Types of Inorganic/Metallic Nanoparticles Used in Pharmaceuticals**

| Nanomaterial Type | Example | Key Properties | Main Pharmaceutical Applications |
|---|---|---|---|
| Inorganic nanoparticles | Silica nanoparticles | High surface area, tunable pore size, stable, biocompatible | Controlled drug release (small molecules, peptides, proteins), imaging, gene delivery |
| | Ceramic nanoparticles (e.g., alumina, titania) | Good mechanical strength, thermal stability, controlled morphology | Carriers for poorly soluble drugs, vaccine adjuvants, tissue engineering scaffolds |
| | Layered double hydroxides (LDHs) | Layered structure, ion-exchange capabilities, pH-responsive release | Controlled release of anionic drugs, gene delivery, stabilizing therapeutic molecules |
| Metallic nanoparticles | Gold nanoparticles (AuNPs) | Surface plasmon resonance, easy surface functionalization, biocompatible | Targeted drug delivery, photothermal therapy, biosensing, imaging contrast agents |
| | Silver nanoparticles (AgNPs) | Antimicrobial properties, tunable size and shape | Antimicrobial coatings, wound dressings, delivery systems against infectious diseases |
| Calcium-based nanoparticles | Polyethyleneimine (PEI)-coated amorphous calcium carbonate (ACC) nanoparticles | High loading capacity due to porous structure, pH-triggered dissolution, enhanced cellular uptake (due to PEI) | pH-responsive drug/gene delivery, intracellular delivery of nucleic acids, controlled release of active agents |
| | Lipid-coated calcium phosphate (LCP) nanoparticles | Biocompatible, biodegradable, enhanced stability and circulation due to lipid coating, pH-sensitive dissolution | Delivery of siRNA, DNA, and small-molecule drugs, gene editing tools, improved intracellular release |
| Carbon-based nanomaterials | Carbon nanotubes (CNTs) | High tensile strength, large surface area, modifiable surfaces, ability to encapsulate drugs | Drug/gene delivery carriers, imaging agents, biosensors for disease detection |
| | Graphene/graphene oxide | High surface area, versatile surface chemistry, strong mechanical properties, photothermal capabilities | Drug and gene delivery, bioimaging, photothermal therapy, biosensors |
| | Fullerenes (C60) | Cage-like structure, antioxidant properties, good electron affinity, modifiable surface | Drug delivery (especially hydrophobic drugs), photodynamic therapy, imaging probes |
| Quantum dots (QDs) | Semiconductor nanocrystals (e.g., CdSe, CdTe, InP) | Size-tunable emission spectra, high brightness, photostability, surface modifiability | *In vitro/in vivo* fluorescent imaging, targeted drug delivery with real-time tracking, multiplexed biosensing |

## 10.2 MICROEMULSION SYNTHESIS

The microemulsion method has been explored as a promising approach for the large-scale synthesis of metallic nanoparticles, including mono- and bimetallic nanoparticles, for pharmaceutical applications [34]. Microemulsions are thermodynamically stable, isotropic dispersions of two immiscible liquids, such as water and oil, stabilized by an interfacial film of surfactant molecules. This unique structure of microemulsions allows for the controlled synthesis of nanoparticles with desired properties.

In general, the microemulsion synthesis of metallic nanoparticles starts with the preparation of the microemulsion, which typically includes the selection of appropriate surfactants, co-surfactants, and oil and aqueous phases to form a stable microemulsion. The desired metal salts (e.g., platinum, palladium, gold, silver) are then added to the microemulsion system. Later, a reducing agent, such as hydrazine or sodium borohydride, is introduced to the microemulsion to reduce the metal ions and facilitate the formation of metallic nanoparticles. Finally, stabilizing agents, such as polymers or surfactants, are added to the microemulsion to prevent the agglomeration of the synthesized nanoparticles. The microemulsion synthesis method has been successfully employed for the large-scale production of various metallic nanoparticles, including platinum-based, palladium-based, and silver-based nanoparticles, which have shown promising applications in the pharmaceutical field, such as catalysis, drug delivery, and antimicrobial therapy [34].

## 10.3 RESONANT ACOUSTIC MIXING (RAM) METHOD

Resonant acoustic mixing (RAM) is an emerging technology that has shown great potential for the large-scale production of inorganic metallic nanoparticles for pharmaceutical applications [35]. This method utilizes high-intensity acoustic fields to achieve highly efficient particle collisions at low frequencies, typically around 60 Hz [36]. The key advantages of using RAM for the synthesis of inorganic metallic nanoparticles are its scalability and the ability to produce nanoparticles with controlled size, shape, and composition [35]. Unlike traditional methods, RAM does not require the use of bulk solvents or milling media, making it a more environmentally friendly and cost-effective approach [37].

Additionally, the RAM method is compatible with various metallic precursors, allowing for the synthesis of a wide range of metallic nanoparticles, including gold, silver, and platinum, among others [37]. This versatility enables the production of tailored nanoparticles to meet the specific requirements of different pharmaceutical applications, such as drug delivery, imaging, and antimicrobial therapy. By tuning the acoustic frequency, acceleration, and other process parameters, researchers can optimize the size, composition, and other characteristics of the metallic nanoparticles synthesized [38]. This level of control is crucial for ensuring the consistent quality and performance of nanoparticle-based pharmaceutical products.

Several studies have demonstrated the successful application of RAM for the synthesis of a wide range of inorganic nanoparticles, including metal-organic frameworks (MOFs) [39], and cocrystals [35]. The ability to readily scale up these reactions without significant changes in reaction conditions is a significant advantage

of the RAM method [37]. In the context of pharmaceutical applications, using RAM to produce lipid-based liquid-crystal nanoparticles, such as those used in mRNA vaccines for COVID-19, has been particularly promising [38]. Furthermore, the combination of RAM with in-line and real-time monitoring techniques, such as near-infrared spectroscopy and chemometric technology, has been explored for process analytical technology (PAT) applications in pharmaceutical powder blending systems [40]. This integration of RAM with advanced analytical tools can help ensure the quality and consistency of the produced nanoparticles, which is crucial for their use in pharmaceutical formulations [40].

The RAM method can also be combined with other techniques, such as liquid-assisted grinding (LAG), to further enhance the efficiency and scope of the mechanochemical synthesis of nanoparticles and other functional materials. The integration of RAM with LAG, referred to as liquid-assisted resonant acoustic mixing (LA-RAM), has been demonstrated to enable the large-scale production of MOFs and other complex nanomaterials [41].

## 10.4 MICROFLUIDICS

Microfluidics has emerged as a promising approach for the large-scale production of metallic nanoparticles with controlled properties for pharmaceutical applications [42, 43]. Compared to traditional batch synthesis methods, microfluidic systems offer several advantages that make them attractive for the scalable manufacturing of metallic nanoparticles.

Microfluidic reactors provide enhanced control over the reaction conditions, such as temperature, residence time, and reagent mixing, leading to improved control over the size, shape, and composition of the synthesized metallic nanoparticles [43–45]. The laminar flow regime in microfluidic devices allows for the production of nanoparticles without the need for steric stabilizing agents, which can simplify the purification and functionalization processes [46]. The ability to parallelize microfluidic devices or use larger-diameter tubing can further increase the production capacity and throughput [47]. Microfluidic platforms can be designed to incorporate in situ functionalization of the synthesized metallic nanoparticles, enabling the direct production of surface-modified nanoparticles for specific pharmaceutical applications [48]. The integration of downstream processing steps, such as purification and separation, can also be achieved within microfluidic systems, further streamlining the overall production process [49].

Several examples of the use of microfluidics for the large-scale production of metallic nanoparticles have been reported in the literature. Pekkari et al. demonstrated the continuous microfluidic synthesis of palladium (Pd) nanocubes and PdPt core-shell nanoparticles, highlighting the potential for the production of bimetallic nanoparticles with controlled properties [44]. Hallot et al. reported the use of a millifluidic continuous-flow process for the synthesis of metallic bismuth nanoparticles, showcasing the robustness and reproducibility of the microfluidic approach [42].

In addition to the advantages mentioned above, the integration of microfluidics with green synthesis approaches, further enhances the sustainability and environmental friendliness of the manufacturing process, making it a valuable tool for the

large-scale production of metallic nanoparticles for pharmaceutical applications. For example, the integration of biological methods, such as the use of plant extracts or microorganisms, can enable the green synthesis of metallic nanoparticles within microfluidic systems [50].

## 10.5 DIRECT DISASSEMBLY-ASSISTED SYNTHESIS (DDAS)

The large-scale production of metallic nanoparticles with well-defined shapes and sizes is a crucial challenge in the field of nanomedicine. Conventional synthesis methods often struggle to achieve the desired control over nanoparticle properties while maintaining scalability. To address this, researchers have developed a novel approach called the Direct Disassembly-Assisted Synthesis (DDAS) strategy [51]. The DDAS method offers a conceptually new approach for the scalable synthesis of polymer/inorganic hybrid nanoparticles. The key aspect of this strategy is the direct disassembly of large, hexagonally packed structures of polypeptide α-helices into small aggregates, which then leads to the formation of the desired nanoparticles [51].

The DDAS process involves incorporating cyanoferrate into polypeptides, which triggers the disassembly of the large, ordered structures of the polypeptide α-helices. This disassembly step is a crucial aspect of the DDAS strategy, as it allows for the formation of tiny nanoparticles with well-defined shapes and sizes [51]. Compared to traditional nanoparticle synthesis methods, the DDAS approach offers several advantages such as scalability, controlled nanoparticles geometry, and versatility for the large-scale production of metallic nanoparticles for pharmaceutical applications. The DDAS method has been demonstrated for the synthesis of polymer/inorganic hybrid nanoparticles, including those containing metallic components. For example, the incorporation of cyanoferrate into polypeptides has been shown to lead to the disassembly of large, hexagonally packed structures and the formation of tiny nanoparticles [51].

## 10.6 MICROWAVE SYNTHESIS

Microwave-assisted synthesis has emerged as a promising approach for the large-scale production of metallic nanoparticles with various applications, including in the pharmaceutical industry. Compared to conventional heating methods, microwave synthesis offers several advantages, such as rapid and uniform heating, reduced reaction times, and improved control over the properties of the synthesized nanoparticles [42, 52, 53]. The rapid and homogeneous heating provided by microwave irradiation can enhance the nucleation and growth processes during nanoparticle synthesis, leading to the formation of nanoparticles with high crystallinity, narrow size distributions, and controlled morphologies [53–55]. This level of control over the nanoparticle properties is crucial for their pharmaceutical applications, where specific characteristics are often required for targeted drug delivery, bioimaging, or antimicrobial activities. One of the key benefits of microwave-assisted synthesis is the ability to produce metallic nanoparticles in a scalable and energy-efficient manner. The rapid heating and reduced reaction times associated with microwave irradiation can enable the large-scale production of nanoparticles while minimizing

energy consumption and processing costs [56, 57]. This is particularly important for the pharmaceutical industry, where the cost-effective manufacture of nanomaterials is a critical factor for their widespread adoption. Moreover, microwave synthesis can be combined with other techniques, such as the use of ultrasound, to further enhance the nanoparticle synthesis process. The combination of microwave and ultrasound treatments has been shown to improve the size control and uniformity of the produced nanoparticles [56]. This synergistic approach can be beneficial for the large-scale production of metallic nanoparticles with tailored properties for pharmaceutical applications. The microwave-assisted synthesis of metallic nanoparticles can also be carried out using green and sustainable methods, which is highly desirable for pharmaceutical applications. By employing plant extracts or other natural reducing and capping agents, the microwave synthesis of silver, gold, and other metallic nanoparticles can be performed in an eco-friendly manner, without the use of harsh chemicals [52, 58, 59]. These green synthesis approaches can lead to the production of biocompatible and environmentally friendly metallic nanoparticles for pharmaceutical use. Furthermore, microwave-assisted synthesis has been successfully applied to producing a wide range of metallic nanoparticles, including gold, silver, copper, and platinum, as well as their alloys and composites [60–62]. The versatility of this method allows for the tailored synthesis of metallic nanoparticles with specific compositions, sizes, and shapes, which can be crucial for their targeted pharmaceutical applications.

## 10.7 GREEN SYNTHESIS USING PLANT EXTRACTS

The large-scale production of metallic nanoparticles for pharmaceutical applications has been a significant challenge, as traditional chemical synthesis methods often involve the use of hazardous reagents and generate toxic byproducts. In recent years, the green synthesis of metallic nanoparticles using plant extracts has emerged as a promising alternative approach that addresses these concerns [63–65]. The green synthesis of metallic nanoparticles using plant extracts is a simple, eco-friendly, and cost-effective method compared to conventional chemical synthesis techniques [64, 66, 67]. This approach utilizes the natural reducing and stabilizing agents present in plant extracts, such as polyphenols, flavonoids, and other phytochemicals, to facilitate the reduction of metal ions and the formation of stable nanoparticles [67–69].

One of the key advantages of the green synthesis approach is its potential for large-scale production. The use of plant extracts as the reducing and capping agents makes the process scalable, as plant materials are readily available, renewable, and can be easily obtained in large quantities [64, 70]. Additionally, the green synthesis method is generally considered safer and more environmentally friendly, as it avoids the use of hazardous chemicals and generates minimal waste [71, 72]. Various types of metallic nanoparticles, including silver (Ag), gold (Au), copper (Cu), and iron oxide ($Fe_3O_4$), have been successfully synthesized using plant extracts [73–75]. The green synthesis process typically involves the following steps (Figure 10.2). The desired part of the plant (e.g., leaves, flowers, fruits, or bark) is collected, cleaned, and subjected to extraction using a suitable solvent, such as water, ethanol, or methanol [76–78]. The plant extract is mixed with a solution containing the metal precursor

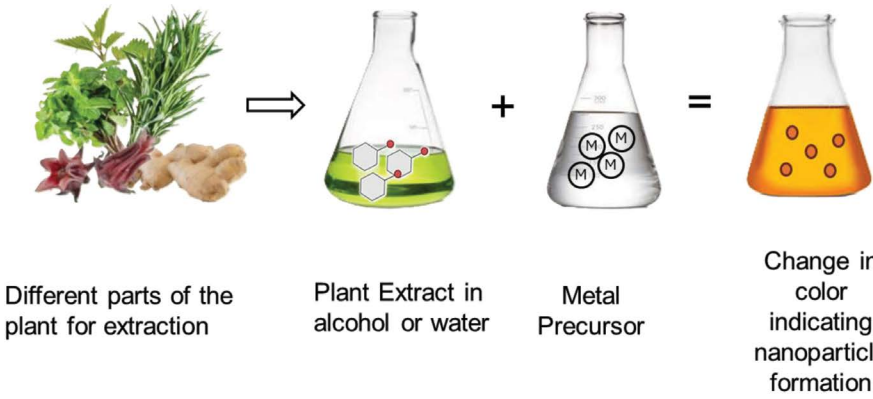


**FIGURE 10.2** Green synthesis of nanoparticles.

(e.g., silver nitrate, gold chloride, copper sulfate, or iron salts). The phytochemicals in the extract act as reducing and capping agents, facilitating the reduction of the metal ions and the formation of stable nanoparticles [79, 80].

The synthesis parameters, such as the plant extract concentration, metal precursor concentration, pH, and reaction time, can be optimized to obtain the desired nanoparticle properties [81, 82]. The green-synthesized metallic nanoparticles have shown promising applications in the pharmaceutical industry, including antimicrobial, anti-inflammatory, and anti-cancer activities [69, 83, 84]. The synergistic effects of the nanoparticles and the phytochemicals present in the plant extracts can enhance the therapeutic efficacy of these nanomaterials [74, 84]. Furthermore, the green synthesis approach offers the potential for large-scale production of metallic nanoparticles for pharmaceutical applications. The use of renewable and readily available plant resources, the simplicity of the synthesis process, and the eco-friendly nature of the method make it an attractive option for the industrial-scale production of these nanomaterials [64, 81].

## 10.8 AQUEOUS TWO-PHASE SYSTEMS (ATPS)

Aqueous two-phase systems (ATPS) have emerged as a promising approach for the large-scale production and simultaneous separation of metallic nanoparticles for pharmaceutical applications [85, 86]. ATPS are liquid-liquid biphasic systems characterized by two immiscible aqueous phases, which can be formed by combining two different polymers or a polymer and a salt.

ATPS allow for the simultaneous synthesis and separation of nanoparticles in a single step, eliminating the need for additional purification steps [85]. This integrated approach can significantly improve the efficiency and scalability of the nanoparticle production process. The components used in ATPS, such as polymers and salts, are generally biocompatible, making the system suitable for pharmaceutical applications [86]. This biocompatibility is crucial for the development of nanoparticle-based DDS and other biomedical applications. The properties of the ATPS, such as the choice

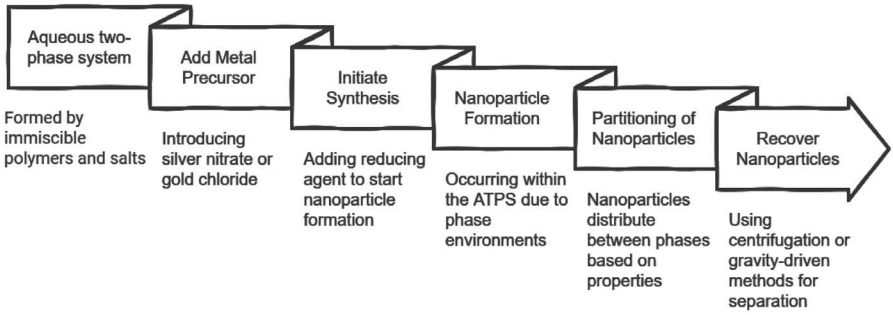


**FIGURE 10.3** ATPS preparation and separation of nanoparticles.

of polymers, salts, and their concentrations, can be tuned to optimize the synthesis and separation of the desired metallic nanoparticles. This flexibility allows for the production of nanoparticles with tailored characteristics, such as size, shape, and composition. The general steps involved in the ATPS synthesis of metallic nanoparticles are shown in the Figure 10.3.

Two immiscible aqueous phases are formed by mixing appropriate concentrations of polymers (e.g., polyethylene glycol, dextran) and/or salts (e.g., sodium citrate, potassium phosphate). The choice of the ATPS components and their concentrations can be optimized to achieve the desired nanoparticle properties. The metal precursor (e.g., silver nitrate, gold chloride) is added to the ATPS, and the nanoparticle synthesis is initiated, typically by the addition of a reducing agent. The nanoparticle formation occurs within the ATPS, taking advantage of the different physicochemical environments in the two phases. The nanoparticles partition selectively between the two phases of the ATPS, depending on their physicochemical properties and the system composition. The nanoparticles are recovered from the desired phase by simple phase separation, such as centrifugation or gravity-driven separation.

The key processing parameters that influence the ATPS synthesis and separation of metallic nanoparticles include polymer and salt types and concentrations, pH, and temperature, reducing agent type and concentration, and reaction time.

## 10.9 CONTINUOUS-FLOW AND MILLIFLUIDIC SYNTHESIS

Continuous-flow and millifluidic synthesis methods have been identified as alternatives to batch reactions for the large-scale production of nanoparticles [44]. These approaches can mitigate the limitations of batch methods, such as inhomogeneities and batch-to-batch variability, by providing excellent mixing, heat and mass transport, and improved control over nucleation and crystal growth [44, 87–89]. This can lead to the production of more monodisperse nanoparticles at a larger scale.

Continuous-flow and millifluidic synthesis have emerged as promising approaches for the large-scale production of metallic nanoparticles for pharmaceutical applications. These methods offer several advantages over traditional batch synthesis techniques, including improved control over nanoparticle properties, enhanced scalability, and the potential for continuous operation.

Continuous-flow synthesis involves the use of microfluidic devices or millifluidic systems to produce metallic nanoparticles in a continuous manner [89–92]. In this approach, the reactants are continuously pumped through a microfluidic channel or a series of interconnected channels, where the nanoparticle formation and growth occur. The continuous-flow nature of the process allows for better control over the reaction parameters, such as residence time, mixing, and temperature, leading to improved control over the size, shape, and composition of the nanoparticles [90]. Continuous-flow synthesis can be particularly advantageous for the production of bimetallic or core-shell nanoparticles, as it enables the precise control over the reaction conditions required for their formation [93]. One key advantage of continuous-flow synthesis is the ability to scale up the production by simply increasing the number of parallel microfluidic channels or by using larger channel dimensions, without compromising the nanoparticle properties [92].

## 10.10 PROCESSING PARAMETERS AND THEIR INFLUENCE

The flow rate of the reactants in the microfluidic or millifluidic system can significantly influence the residence time, mixing, and ultimately, the size and morphology of the nanoparticles [94]. The residence time of the reactants in the microfluidic or millifluidic system determines the duration of the nanoparticle formation and growth processes, affecting the final nanoparticle characteristics [95].

The concentration of the metal precursors and reducing agents can affect the nucleation and growth kinetics, leading to changes in the nanoparticle size and size distribution [96]. The reaction temperature can impact the reaction kinetics, solubility, and diffusion rates, which in turn can influence the nanoparticle properties, such as size, shape, and crystallinity [96]. Efficient mixing of the reactants is crucial for nanoparticle homogeneous nucleation and growth. The channel geometry, flow patterns, and mixer designs can all contribute to the mixing efficiency [97].

## 10.11 SUPERCRITICAL FLUID SYNTHESIS

Supercritical water synthesis has been explored for the large-scale production of surface-functionalized nanoparticles, such as cerium-zinc oxide nanoparticles [98]. This method can enable the continuous synthesis of nanoparticles with controlled properties and surface functionalization. Supercritical fluid reactors can be designed for continuous, high-throughput production of metallic nanoparticles, making the process suitable for industrial-scale manufacturing.

This method leverages the unique properties of supercritical fluids, such as their gas-like diffusivity, low viscosity, and high solvation capability, to enable the controlled synthesis of nanoparticles with desirable characteristics. The advantages of using supercritical fluid synthesis for the large-scale production of metallic nanoparticles for pharmaceutical applications include controlled nanoparticle properties and improved purity and crystallinity, being environmentally friendly, and reducing the environmental impact compared to traditional chemical synthesis methods.

In the supercritical fluid synthesis process, a metal precursor, such as a metal salt or organometallic compound, is dissolved in a supercritical fluid, typically

supercritical carbon dioxide ($scCO_2$) or supercritical water ($scH_2O$). The supercritical fluid acts as both the solvent and the reaction medium, providing a highly reactive environment for the formation and growth of the metallic nanoparticles.

The key steps involved in the supercritical fluid synthesis of metallic nanoparticles are as follows. The metal precursor is dissolved in the supercritical fluid, which can be either $scCO_2$ or $scH_2O$, depending on the specific application and the properties of the desired nanoparticles. The precursor solution is rapidly mixed with an anti-solvent or a reducing agent, such as hydrogen gas, to initiate the nucleation and growth of the metallic nanoparticles within the supercritical fluid environment [99]. The high temperature and pressure conditions, as well as the unique properties of the supercritical fluid, promote the rapid nucleation and controlled growth of the metallic nanoparticles, leading to the formation of nanoparticles with desired size, shape, and composition. The synthesized nanoparticles are then separated from the supercritical fluid, typically through a rapid depressurization or cooling process, and collected for further processing or application.

The key processing parameters that influence the characteristics of the metallic nanoparticles produced by supercritical fluid synthesis include:

- Temperature and pressure: The temperature and pressure of the supercritical fluid can be tuned to control the solubility, reaction kinetics, and the resulting nanoparticle properties.
- Precursor concentration: The concentration of the metal precursor in the supercritical fluid can affect the nucleation and growth rates, as well as the final nanoparticle size and distribution.
- Mixing and residence time: The efficiency of mixing and the residence time of the reactants in the supercritical fluid environment can impact the nanoparticle morphology and size.
- Reducing agent and anti-solvent: The choice and concentration of the reducing agent or anti-solvent can influence the reduction and precipitation of the metallic nanoparticles.

However, the supercritical fluid synthesis of metallic nanoparticles also faces some challenges, such as the need for specialized high-pressure equipment, the potential for scale-up issues, and the potential for residual solvent or impurities in the final nanoparticle products, which must be carefully addressed to ensure the safety and efficacy of the pharmaceutical applications.

## 10.12 QUANTUM DOTS

QDs are semiconductor nanocrystals that exhibit size-dependent optical and electronic properties, making them attractive for bioimaging, biosensing, and drug delivery applications in the pharmaceutical field [100].

One of the prominent methods for the large-scale synthesis of QDs is the colloidal synthesis approach, which includes techniques like hot-injection and heat-up methods [101]. These colloidal synthesis methods allow for the tuning of QD properties, such as size and composition, by carefully controlling the reaction conditions,

such as temperature, precursor concentrations, and reaction time. The hot-injection method, in particular, has been widely used for the production of high-quality semiconductor QDs, including cadmium selenide (CdSe) and indium phosphide (InP) QDs, which are of great interest for biomedical applications [101].

Another approach that has been explored for the large-scale production of QDs is the use of microfluidic platforms [102]. Microfluidic systems offer several advantages for the synthesis of QDs, including improved control over the reaction conditions, the potential for continuous production, and the ability to scale up the process. By leveraging the precise control and mixing capabilities of microfluidic devices, researchers have demonstrated the feasibility of producing QDs with tunable properties and high quality.

In addition to the colloidal and microfluidic synthesis methods, biological approaches have also been investigated for the large-scale production of QDs in a more sustainable and eco-friendly manner [103]. These biological synthesis methods involve the use of microorganisms, such as bacteria or fungi, or plant extracts to facilitate the formation of QDs. The biomolecules present in these biological systems can act as reducing and capping agents, enabling the synthesis of QDs with improved biocompatibility, which is particularly important for pharmaceutical applications.

The choice of the large-scale production method for QDs in the pharmaceutical field depends on various factors, such as the desired QD properties, the target application, and the need for scalability and cost-effectiveness. Each method offers its own advantages and challenges, and researchers continue to explore innovative strategies to optimize the synthesis, purification, and functionalization of QDs to meet the specific requirements of the pharmaceutical industry.

Regardless of the production method, the large-scale synthesis of QDs must also address important considerations, such as the potential toxicity of the QD materials, particularly those containing heavy metals like cadmium, and the need for effective surface passivation and functionalization to enhance their biocompatibility and targeting capabilities [104, 105]. Ongoing research in this field aims to develop safer and more sustainable QD materials, as well as to streamline the large-scale production processes to enable the widespread adoption of QDs in pharmaceutical applications.

## 10.13 SYNTHESIS OF CARBON-BASED NANOMATERIALS

Carbon-based nanomaterials, such as CNTs and graphene, have garnered significant attention in the pharmaceutical field due to their unique physicochemical properties and potential applications. CNTs have been extensively investigated for pharmaceutical applications, including drug delivery, tissue engineering, and biosensing, due to their unique structural and electronic properties [106].

One of the most widely used methods for the large-scale synthesis of CNTs is chemical vapor deposition (CVD). In the CVD process, a hydrocarbon gas, such as methane or ethylene, is decomposed at high temperatures (typically 600–1200°C) in the presence of a metal catalyst, such as iron, cobalt, or nickel. The catalyst nanoparticles act as nucleation sites for the growth of CNTs, which can be either single-walled (SWCNTs) or multi-walled (MWCNTs). The large-scale production of CNTs has been achieved through various methods, with CVD being a widely used

technique. The CVD process can be optimized to produce CNTs, both SWCNTs and MWCNTs, with controlled properties, such as diameter and length, by manipulating the experimental conditions. By carefully controlling the reaction parameters, such as temperature, gas flow rate, and catalyst composition, the properties of the synthesized CNTs, including their diameter, length, and degree of alignment, can be tailored [107].

Another high-temperature method for the large-scale synthesis of CNTs is the arc discharge technique [108]. In this approach, an electric arc is struck between two graphite electrodes in an inert gas atmosphere, typically helium or argon. The intense heat generated by the arc causes the sublimation of the graphite, leading to the formation of CNTs, which can be collected from the chamber. The arc discharge method can produce high-purity CNTs, but the process typically requires more complex setups and higher energy inputs compared to CVD [107].

Laser ablation is a third method that has been explored for the large-scale production of CNTs [108]. In this technique, a high-intensity laser beam is used to vaporize a graphite target in the presence of a catalyst, such as cobalt or nickel. The vaporized carbon then condenses to form CNTs, which can be collected from the chamber. Laser ablation can produce high-quality CNTs, but the process is generally more expensive and less scalable compared to CVD.

In addition to the traditional methods, researchers have also explored catalyst-free approaches for the large-scale synthesis of CNTs. These methods involve the direct growth of CNTs on cheap and simple substrates, such as silicon or quartz, without the use of external catalysts. The elimination of metal catalysts can lead to the production of relatively pure CNTs that are free of metal oxide residues, which can be beneficial for certain pharmaceutical applications [109].

The large-scale production of CNTs for pharmaceutical applications often requires additional steps for functionalization and purification. For example, the use of KOH etching has been reported to remove amorphous carbon and trace heavy metals from CNT samples, improving their biocompatibility and suitability for biomedical applications [110].

Regarding the large-scale production of other carbon-based nanomaterials, such as graphene, the methods are still evolving, and significant research efforts are ongoing to develop scalable and cost-effective synthesis approaches. Some promising techniques include chemical exfoliation, thermal reduction of graphene oxide, and CVD on metal substrates. Notably, the large-scale production of carbon-based nanomaterials, particularly for pharmaceutical use, must also address concerns related to their potential toxicity and environmental impact. Careful characterization, surface modification, and comprehensive safety assessments are crucial steps to ensure the biocompatibility and responsible development of these nanomaterials for biomedical applications.

Table 10.3 summarizes the key features and advantages of the different large-scale production methods for inorganic and metallic nanoparticles, CNTs, and QDs.

These large-scale production methods offer various advantages, such as improved control over nanoparticle properties, scalability, cost-effectiveness, and environmental friendliness, making them suitable for the large-scale manufacturing of inorganic and metallic nanoparticles, CNTs, and QDs for pharmaceutical applications.

**TABLE 10.3**
**Key Features and Advantages of the Different Large-Scale Production Methods for Inorganic/Metallic Nanoparticles**

| Production Method | Key Features | Advantages |
|---|---|---|
| Microemulsion synthesis | • Use of water-in-oil or oil-in-water microemulsions as nanoreactors<br>• Control over composition and size of nanoparticles | • Ability to produce mono- and bimetallic nanoparticles<br>• Potential for scalability |
| Resonant acoustic mixing (RAM) | • Use of high-frequency acoustic waves to mix components<br>• Production of lipid-based liquid-crystal nanoparticles | • Scalability<br>• Ability to produce nanoparticles with controlled properties |
| Microfluidics | • Excellent mixing, heat and mass transport<br>• Improved control over nucleation and crystal growth | • Production of more shape- and size-uniform nanoparticles<br>• Mitigation of limitations associated with batch methods |
| Shape-controlled synthesis | • Manipulation of shape and defect structures of nanocrystals | • Ability to tailor the catalytic properties of nanoparticles |
| Direct disassembly-assisted synthesis (DDAS) | • Direct disassembly of large hexagonally packed structures into small aggregates | • Scalable production of polymer/inorganic hybrid nanoparticles with well-defined shapes and sizes |
| Rapid, high-temperature, in situ microwave synthesis | • Use of high-temperature, in situ microwave heating | • Simple, efficient, and scalable production of nanocatalysts |
| Green synthesis using plant extracts | • Use of plant extracts and other bio-based reducing agents | • Cost-effective, environmentally friendly, and safe for human therapeutic use |
| Aqueous two-phase systems (ATPS) | • Simultaneous synthesis and separation of nanoparticles | • Environmentally benign production and purification of nanoparticles |
| Continuous-flow and millifluidic synthesis | • Excellent mixing, heat and mass transport<br>• Improved control over nucleation and crystal growth | • Production of more monodisperse nanoparticles at a larger scale<br>• Mitigation of limitations associated with batch methods |
| Supercritical fluid synthesis | • Continuous synthesis in supercritical water | • Production of surface-functionalized nanoparticles with controlled properties |

## 10.14 CHARACTERIZATION OF INORGANIC/METALLIC NANOPARTICLES

Inorganic and metallic nanoparticles, QDs, and carbon-based nanomaterials, including CNTs, can be characterized using various methods and tools. Comprehensive characterization of these nanoparticles is crucial to ensure their safety, efficacy, and suitability for biomedical applications.

One of the primary characterization techniques for inorganic/metallic nanoparticles is dynamic light scattering (DLS), which provides information on the size distribution and zeta potential of the nanoparticles [111]. Taylor dispersion analysis (TDA) was also used to characterize the diffusion coefficient and the hydrodynamic radius of nanoparticles [112]. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are also widely used to analyze the morphology, size, and structure of the nanoparticles [113]. Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) can be employed to investigate the chemical composition and crystalline structure of the nanoparticles [114].

For nanoparticles intended for drug delivery applications, additional characterization methods are essential. The drug loading capacity, drug release kinetics, and stability of the nanoparticles in biological media can be evaluated using techniques such as UV-visible spectroscopy, high-performance liquid chromatography (HPLC), and *in vitro* drug release studies [115].

Magnetic nanoparticles, such as iron oxide ($Fe_3O_4$) nanoparticles, are of particular interest for biomedical applications due to their unique magnetic properties. These nanoparticles can be characterized using magnetometry techniques, such as vibrating sample magnetometry (VSM) or superconducting quantum interference device (SQUID) magnetometry, to determine their magnetic properties [116, 117].

For nanoparticles intended for targeted drug delivery or diagnostic applications, the surface functionalization and targeting capabilities are crucial. Techniques like zeta potential measurements, X-ray photoelectron spectroscopy (XPS) [118], and cell-based assays [119] can be used to evaluate the surface properties, targeting ligand conjugation, and cellular uptake of the nanoparticles.

Additionally, the biocompatibility and potential toxicity of inorganic/metallic nanoparticles must be thoroughly assessed. *In vitro* cytotoxicity assays, such as MTT or LDH assays, and *in vivo* studies using animal models can be employed to evaluate the biocompatibility and potential adverse effects of the nanoparticles [120–122].

CNTs have been extensively studied and characterized using various techniques. Luca et al. have characterized non-purified (CNTs UP), purified (CNTs P), and oxidized (CNTs OX) CNTs, reporting their thermal characteristics [123]. Ghann et al. have used terahertz reflectometry imaging and Raman spectroscopy to characterize MWCNTs [124]. Siddheswaran et al. have used XRD to characterize the structure and orientation of CNTs [125]. Engels et al. have fabricated and characterized all-carbon hybrid quantum devices based on graphene and SWCNTs [126].

Nondestructive evaluation techniques, such as magnetic characterization, Raman scattering, and polarized Raman scattering, have been applied to characterize the properties of CNT-based materials [127]. A review of the recent advancements in CNTs and their composites, including their characterization and modelling is available [128]. Raman spectroscopy has also been extensively used to characterize CNTs and graphene, as discussed by Dresselhaus et al. [129].

Graphene and its derivatives have also been characterized using techniques such as XRD [130]. Polarized light microscopy and spectroscopy have been used to image and characterize individual SWCNTs [131]. Capillary electrophoresis has been used to characterize the surface oxidation and carboxylation of MWCNTs [132]. Li et al. have studied the surface charge and cellular processing of functionalized CNTs

[133]. A novel on-chip Rayleigh imaging and spectroscopy technique to measure the optical scattering from individual SWCNTs, which can be used to determine their electronic types, chiral indices, and intertube interactions, was reported [134].

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# 11 Scaleup of Nano/ Micro Emulsions

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## 11.1 INTRODUCTION

Nanoemulsions are kinetically stable liquid-in-liquid dispersions with droplet sizes between 20 and 500 nm that require high energy to be efficiently created. At the same time, microemulsion is a thermodynamically stable, clear or translucent system consisting of two immiscible liquids (typically oil and water) stabilized by surfactants and, often, cosurfactants. These systems form spontaneously and are characterized by droplet sizes typically in the range of 10–100 nanometers. Micro/nanoemulsion can increase the solubility of insoluble and fat-soluble drugs and load water-soluble drugs, extend the action time, improve the bioavailability of drugs, and exert sustained release and targeted effects. With the rapid development of nanotechnology, micro/nanoemulsions are increasingly used in various industries.

## 11.2 ADVANTAGES OF MICRO/NANOEMULSIONS

- Improve the bioavailability of drugs.
- Non-toxic and non-irritating, depending on the composition used.
- Small-sized droplets with larger surface area, providing greater absorption.
- Can be formulated into various dosage forms, including foams, creams, liquids, and sprays.
- Help dissolve lipophilic drugs.
- Help mask taste.

Microemulsions are usually manufactured using low-energy methods due to their thermodynamic stability. At the same time, nanoemulsions are generally formulated using high-energy methods that require the use of high-shear devices to increase the water/oil interfacial area for generating sub-microscopic droplets and kinetically stable emulsions. Self-emulsifying drug delivery systems (SEDDS) are often marketed as microemulsion preconcentrates which form microemulsions upon contact with aqueous fluids in the body. Table 11.1 provides details of marketed nano/microemulsion formulations for pharmaceutical use.

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**TABLE 11.1**
**Marketed Nano/Microemulsions**

| Product Name and Manufacturer | Emulsion Type | Application Area | Key Active/ Ingredients | Typical Composition* |
|---|---|---|---|---|
| Neoral® (Novartis) | Microemulsion | Immunosuppressant (oral) | Cyclosporine A | Cyclosporine, ethanol, corn oil, polyoxyethylated glycerides |
| Cleviprex® (**Chiesi**) | Nanoemulsion | Hypertension | Clevidipine | Clevidipine butyrate, soybean oil, glycerin, purified egg yolk, phospholipids, water |
| Estrasorb®# (Novavax) | Microemulsion | Hormone-replacement therapy | Estradiol, sorbitan monooleate, polysorbate 80, water | Estradiol, sorbitan monooleate, polysorbate 80, water |
| Sandimmune® (Novartis) | Microemulsion preconcentrate | Immunosuppressant (Oral) | Cyclosporine A | Cyclosporine, olive oil, Cremophor® EL, ethanol |
| Fortovase® (Roche) | Microemulsion | Antiretroviral (oral) | Saquinavir mesylate | Saquinavir, soybean oil, vitamin E TPGS, polysorbate 80 |
| Restasis® (Allergan) | Nanoemulsion | Ophthalmic (dry eye) | Cyclosporine | Castor oil, glycerin, polysorbate 80, carbomer, purified water |
| Diprivan® (AstraZeneca) | Nanoemulsion | Anesthetic (IV) | Propofol | Soybean oil, egg lecithin, glycerol |
| NutriNano CoQ10 (SourceOne) | Nanoemulsion | Nutraceutical (oral) | Coenzyme Q10 | Lipid carriers (MCT oil), surfactants (e.g., polysorbates) |

* Exact composition details and surfactant types may vary; listed components are representative.
\# Discontinued.

## 11.3 COMPONENTS AND THEIR SELECTION

**Oil Phase**: Oils serve as the solvent for lipophilic drugs and influence droplet size and drug release rates. Common oils include medium-chain triglycerides, such as caprylic/capric triglycerides, and long-chain triglycerides, like soybean oil. The choice depends on the drug's solubility, the oil's biocompatibility, and its digestibility.

**Aqueous Phase**: Typically, purified water or buffer solutions, the aqueous phase affects the overall stability and tonicity of the formulation.

**Surfactants**: Surfactants reduce interfacial tension (IFT) between the oil and water phases, stabilizing the emulsion. Conventional surfactant molecules comprise a polar head group region and an apolar tail region. On dispersal in water,

surfactants self-associate into various equilibrium phases, the nature of which stems directly from the interplay of the various intermolecular forces and entropy considerations. The surfactants stabilizing such systems could be nonionic, zwitterionic, cationic, or anionic surfactants. However, nonionic surfactants like polysorbates (e.g., Tween 80) and sorbitan esters (e.g., Span 80) are commonly used due to their lower toxicity. Phospholipids are a notable example of zwitterionic surfactants and exhibit excellent biocompatibility. They are also used.

The selection of surfactants is guided by the hydrophile–lipophile balance (HLB) value, which determines the surfactant's affinity toward the oil or water phase [1]. The HLB system assigns a value (on a scale of 0–20) to surfactants based on their affinity for oil or water. Surfactants with low HLB values (3–6) are lipophilic and favor water-in-oil (W/O) emulsions, while those with high HLB values (8–18) are hydrophilic and favor oil-in-water (O/W) emulsions. Selecting a surfactant with an appropriate HLB value is essential for forming a stable emulsion. For microemulsions, a blend of surfactants may be used to achieve the desired HLB. The surfactant molecules position themselves in such a way to minimize the free energy of the system by exposing the hydrophilic regions to the aqueous environment and tightly packing their hydrophobic domains in the interior to minimize the interface with the existing solvent. Surfactant number or critical packing parameter (CPP) is a theoretical framework for determining the type of aggregation formed by surfactants [2]. The equation defines CPP is:

$$\mathbf{CPP} = \frac{\mathbf{v}}{\mathbf{l}.\mathbf{a_0}}$$

V: Volume of hydrocarbon core
L: Hydrocarbon chain length
$a_0$: Effective head group area

CPP predicts the shape of the aggregate formed by surfactants: **CPP < 1/3**: forms spherical micelles, **1/3 < CPP < 1/2** forms cylindrical micelles, **1/2 < CPP < 1** forms vesicles or lamellar structures, **CPP > 1** forms inverted structures like reverse micelles (Figure 11.1). By manipulating the CPP through surfactant selection and combining different surfactants, formulators can control the type of nanostructures formed, which is crucial for the stability and drug delivery performance of nanoemulsions and microemulsions [3].

Understanding both HLB and CPP allows for the rational design of nanoemulsion and microemulsion systems [4, 5]. The proper balance ensures that the surfactants will arrange themselves at the oil-water interface to stabilize the desired emulsion and facilitate drug incorporation and release.

Cosurfactants are also amphiphilic with an affinity for both the oil and aqueous phases and partitions to an appreciable extent into the surfactant interfacial monolayer present at the oil-water interface. The cosurfactant need not necessarily be capable of forming association structures in its own right. Various molecules can function as cosurfactants, including nonionic surfactants, alcohols, alkanoic acids, alkanediols, and alkyl amines. Cosurfactants like short-chain alcohols (e.g., ethanol) or glycols enhance fluidity and reduce IFT further, aiding in the formation of nano/microemulsions [6].

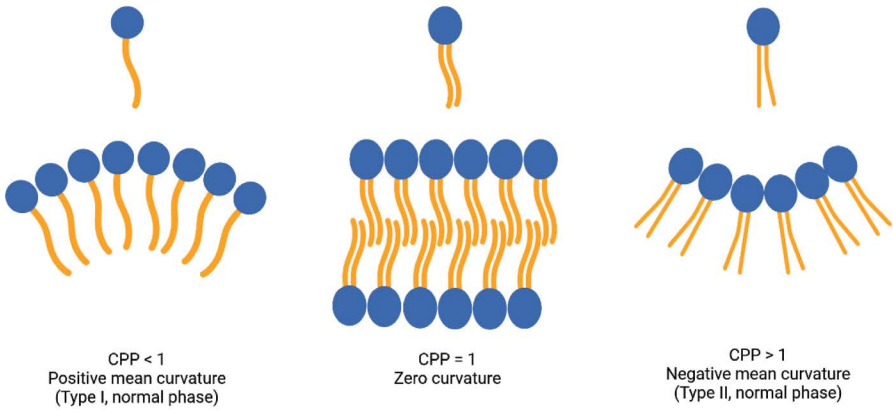


**FIGURE 11.1** Critical Packing Parameters (CPP) of Surfactants.

## 11.4 FORMULATION SCREENING

Formulation screening is the key to preparing nanoemulsions with stable, minimum particle size, minimum polydispersity coefficient, and specific functions. The screening is done to determine the type of oil phase, emulsifier, co-emulsifier, and the optimal ratio with the water phase [6]. In case of improper ingredient selection, the nanoemulsion cannot be formed, or the formed nanoemulsion area is too small and cannot achieve the purpose of solubilizing the drug. As long as the formulation of microemulsion is appropriately selected, microemulsion can be formed spontaneously, regardless of the order in which the ingredients are added. For example, a low-energy emulsification method is used under constant temperature stirring, and water is added dropwise to a mixed system containing emulsifiers, co-emulsifiers, and oil. The system becomes transparent instantly to form microemulsion.

## 11.5 PRODUCTION METHODS

**Low-energy methods**: Spontaneous emulsification, phase-inversion temperature (PIT), and phase inverse composition methods.

**High-energy methods**: High-pressure homogenization, microfluidization, ultrasonication.

High-energy methods use external energy input to disrupt emulsion into nano-sized droplets, while low-energy methods exploit the physicochemical properties of the oil/water/surfactant mixture to form nanoemulsion.

## 11.6 LOW-ENERGY PRODUCTION METHODS

Low-energy methods provide an economical and gentle approach to producing nanoemulsions, protecting sensitive compounds and offering scalability potential. Low-energy techniques do not involve external mechanical force for producing nanoemulsions and mainly depend on the physicochemical properties of the components,

such as oil and surfactants, to facilitate spontaneous formation. These methods, which bypass the need for high shear, pressure, or temperature, create mild conditions ideal for sensitive components and are energy-efficient for large-scale production. Low-energy methods offer a cheap and easy way to produce nanoemulsions. They provide mild conditions to protect sensitive compounds during production. They also have scaleup potential because the emulsification process mainly depends on the physicochemical properties of components rather than external forces.

## 11.7 SPONTANEOUS EMULSIFICATION

Spontaneous emulsification is a straightforward method involving mixing oil, surfactant, water, and additives under specific conditions. A sudden drop in IFT causes the rapid dispersion of the oil phase into the aqueous phase, with surfactants stabilizing the droplets to form a kinetically stable nanoemulsion.

To understand spontaneous emulsification, it is essential to understand the emulsion system's thermodynamics (Gibbs free energy). If two components are completely miscible, they do not form an interface like water and alcohol; the free energy of mixing is negative. Preparing emulsions that involve the dispersion of two immiscible liquids require energy to disperse one phase (such as the organic phase) as fine droplets in another phase (such as water). Gibbs free energy ($\Delta G$) is a thermodynamic potential measuring the maximum reversible work a system can perform at constant temperature and pressure. For a process to occur spontaneously, the change in $\Delta G$ must be negative [7].

In the context of emulsification, Gibbs free energy of emulsification refers to the change in Gibbs free energy when two immiscible liquids (like oil and water) form an emulsion, which is a fine dispersion of one liquid in another. The emulsification process requires energy to create a large interfacial area between two immiscible liquids. The Gibbs free energy change ($\Delta G$) during emulsification is described as:

$$\Delta \mathbf{G} = \Delta \mathbf{H} - \mathbf{T}\Delta \mathbf{S} = \gamma\, \Delta \mathbf{A} - \mathbf{T}\, \Delta \mathbf{S}$$

where $\Delta S$ represents the change in entropy, accounts for the shift in disorder or randomness in the system, is a measure of the extent of disorder in the system, and hence measures the extent of size reduction of the organic phase (or increase in droplet number). $\Delta H$ is the change in enthalpy associated with creating a new interfacial area and the interactions between molecules at the interface or simply the energy input needed to achieve a certain average droplet size. T is the absolute temperature.

Mixing two liquids generally increases the system's disorder, contributing positively to $\Delta S$. Creating a new interface between two immiscible liquids increases the IFT, which increases $\Delta H$. Surfactants can lower the IFT, reducing $\Delta H$. If the interaction between surfactants and the liquids results in lower-energy states, $\Delta H$ can be minimized. Surfactants are often used to lower the IFT ($\gamma$) and increase the entropy change ($\Delta S$) by creating a more disordered system, thus making the emulsification process spontaneous ($\Delta G < 0$). Higher temperatures also increase entropy ($T\Delta S$), favoring spontaneous emulsification. In summary, for spontaneous emulsification to occur, the Gibbs free energy change must be negative, meaning the process results in

a net release of free energy, making it thermodynamically favorable. This is typically achieved by reducing IFT and/or increasing the system's entropy.

For spontaneous emulsification, the Gibbs free energy change must be negative. This means either the enthalpy change must be sufficiently negative, the entropy change must be sufficiently positive, or a combination of both. Surfactants reduce the IFT and often lead to a positive entropy change by dispersing oil droplets within water (or vice versa) and preventing them from coalescing. Concerning the effect of $\Delta A$, O/W microemulsion is likely to occur where the volume fraction of oil is low and vice versa. In systems where the amounts of water and oil are similar, a bicontinuous microemulsion may result in which both oil and water exist as a continuous phase in the presence of a surfactant-stabilized interface with a net curvature of zero.

Ternary and pseudo-ternary phase diagrams help design nanoemulsion systems, indicating the optimal composition ranges for desirable properties. This method can also be adapted to formulate gels, emulsions, and microemulsions. A ternary-phase diagram represents the phase behavior of three distinct components without any grouping or simplifications, while a pseudo-ternary phase diagram simplifies the representation by grouping multiple components (e.g., surfactant and cosurfactant) into a single "pseudo-component."

## 11.8 TERNARY AND PSEUDO-TERNARY PHASE DIAGRAMS

The type, concentration, and ratio of oil, surfactant, and water can significantly influence nanoemulsion properties. Ternary-phase diagrams are used to determine the optimum ratios of a ternary blend. These are equilateral triangles; each side of the triangle corresponds to one component (oil, surfactant, or water) and is divided into 100 equal parts. The total mass of the ternary blend is considered 100%, and the mass of each component is calculated as a fraction of the weight percentage of the total [8]. The corners typically represent either a single, that is, oil, surfactant, and aqueous phase, or a binary mixture of two components, surfactant/cosurfactant, water/drug, or oil/drug, if the formulation components are more than three (Figure 11.2a). To plot a ternary blend, the mass fraction of the components should be calculated as a percentage of the weight [8]. Every spot on the diagram represents a different

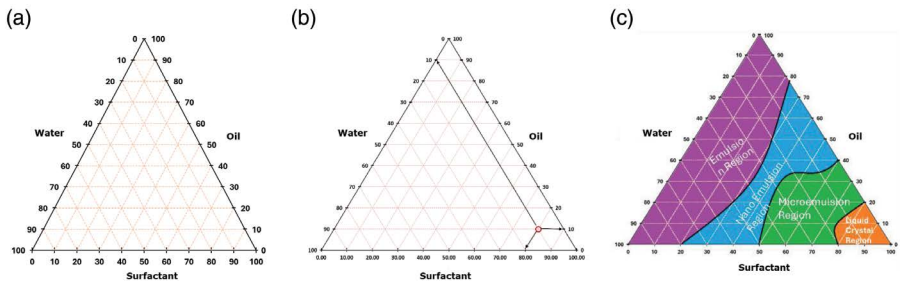


**FIGURE 11.2** Ternary/pseudoternary phase diagrams to determine different phases. (a) Ternary phase diagram,(b) shows the identification of spots based on the fraction weight percentage of each component. (c) Identifying different phases on the diagram.

composition of ternary components, and the mass fractions are read off through the sides of the triangle. The arrows in Figure 11.2b correspond to components of the spot (10% oil, 80% surfactant, and 10% water).

Identifying different regions such as liquid crystals, emulsions, nanoemulsions, and microemulsions on a ternary-phase diagram is essential for understanding the phase behavior of multiphase systems composed of water, oil, and surfactant. The ternary-phase diagram is a graphical representation that illustrates the equilibrium between these three components over various compositions, providing valuable insights into the formulation and optimization of various colloidal systems. To accurately map the phase diagram, it is important to divide it into a grid by selecting systematic increments of component concentrations to cover the entire composition range. Maintaining constant temperature and pressure during the sample preparations and allowing samples to reach equilibrium is crucial, as these factors significantly influence phase behavior [9].

A combination of analytical methods is employed to characterize each sample. The data obtained from the characterization of ternary mixtures are used to classify and map phases (Table 11.2).

Each sample's phase is plotted on the ternary diagram at its corresponding composition. Connecting samples of the same phase delineates phase boundaries, effectively mapping the different regions (Figure 11.2c). Emulsions are identified by turbidity, larger droplet sizes, and potential phase separation and appear where surfactant concentration is insufficient for stabilizing small droplets, resulting in larger, unstable emulsions. Microemulsions are thermodynamically stable, transparent, and form spontaneously due to favorable free energy conditions. Microemulsion regions are found at specific ratios where surfactant facilitates spontaneous mixing of oil and

**TABLE 11.2**
**Characterization of Ternary Mixtures to Identify Distinct Phases**

| Technique | Observation | Interpretation |
|---|---|---|
| Visual observation | Transparent | Microemulsions |
| | Turbid | Emulsions or phase separation |
| | Phase separation | Unstable emulsions |
| Electrical conductivity | High conductivity | Oil-in-water (O/W) |
| | Low conductivity | Water-in-oil (W/O) |
| Viscosity profiling | High viscosity | Liquid crystals |
| | Low viscosity | Emulsions |
| Polarized light microscopy | Birefringence | Liquid crystalline phases (isotropic phases remain dark upon rotation, whereas anisotropic phases vary in brightness) |
| Particle size analysis using dynamic light scattering | Droplet sizes 20–200 nm | Nanoemulsion |
| | Droplet sizes >200 nm | Emulsion |
| Small-angle X-ray, scattering (SAXS), small-angle neutron scattering (SANS) | Distinct scattering patterns indicative of their unique structures | Microemulsion or liquid crystals |

water. Nanoemulsions are kinetically stable, translucent systems with nanosized droplets requiring external energy for formation. Nanoemulsion regions may overlap with microemulsion regions but are distinguished by their formation methods and kinetic stability. Liquid crystals are anisotropic, display birefringence, and possess ordered mesophases, leading to high viscosity and unique rheological properties. Liquid crystal regions typically occur at high surfactant concentrations, forming structured phases with water or oil.

## 11.9 PHASE-INVERSION METHODS

Phase-inversion methods involve changing the system's temperature or composition (Figure 11.3). The PIT method uses temperature adjustment, employing thermal-sensitive surfactants like polyethoxylated nonionic surfactants. With temperature changes, surfactants' hydrophobicity increases due to dehydration, allowing the formation of W/O and O/W emulsions at different temperature regions. The HLB temperature, where transitional phase inversion occurs, enables the formation of microemulsions or liquid crystalline phases, and mild stirring can produce nanoemulsions. Rapid cooling or heating prevents coalescence, ensuring kinetic stability. The PIT method's flexibility is a significant advantage, with adjustments possible multiple times, and NaCl can lower the HLB temperature. Novel thermal-sensitive surfactants are also being developed for PIT nanoemulsion production [10].

Over the past few decades, the PIT method has received much more attention than high-energy emulsification methods because it generates more uniform and smaller droplets without requiring sophisticated devices. In the PIT method, the temperature can dramatically affect the spontaneous curvature of the surfactant layer of temperature-sensitive surfactants. At low temperatures, temperature-sensitive surfactants

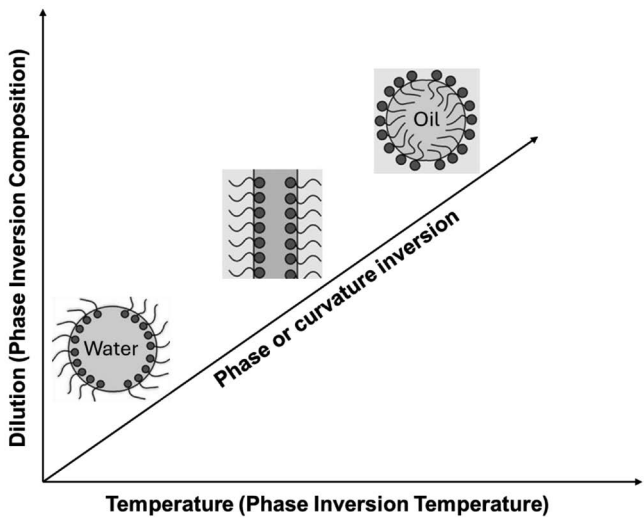


**FIGURE 11.3** Phase Inversion. Change in composition (or dilution) (in PIC) or temperature (in PIT).

are water soluble, and the curvature of the surfactant layer at the droplet interface is positive. In contrast, at high temperatures, the surfactants are oil soluble, and the curvature of the surfactant layer at the droplet interface becomes negative [11]. At an intermediate temperature, known as the PIT, the surfactants have the same affinity for the aqueous and oil phases. As a result, the spontaneous curvature of the surfactant layer at the droplet interface is zero, leading to complete solubilization of oil in a bicontinuous phase or a lamellar liquid crystalline phase [10]. More importantly, the oil-water IFT at the PIT is ultra-low ($10^{-5}$–$10^{-2}$ mN/m), which is favorable for the formation of nanometer-sized droplets [12]. Rapid cooling of samples from the PIT to room temperature affords kinetically stable O/W nanoemulsions.

The phase-inversion composition (PIC) method dilutes the dispersed phase with the continuous phase at a constant temperature until the final composition is achieved. This method is also known as catastrophic phase-inversion (CPI) or emulsion-inversion point method. In PIC, one of the components, such as water, is added to an oil-surfactant mixture, and oil is added to the water-surfactant mixture. Surfactants containing polyoxyethylene (POE) chains are widely used in the PIC method to formulate nanoemulsions due to their composition sensitivity [13]. When water is added slowly to the oil phase and as the volume of the water fraction increases, surfactant POE chain hydration occurs. The surfactant hydrophilic-lipophilic properties of the water phase will become balanced, and the spontaneous curvature of the surfactant will change to zero, like at the HLB temperature in the PIT method. During this transition, a bicontinuous or lamellar structure is formed. When additional water is added the transition composition is exceeded, and the structures of the surfactant layer with zero curvature change to having high positive curvature. This change in curvature leads to phase inversion and causes nanosized droplet formation. Thus, changing the composition of the system causes phase inversion [14, 15]. Similarly, other composition parameters, such as adding salt and pH changes, cause nanosized emulsion droplets by transitional phase inversion [16]. Gradually adding the aqueous phase to the oil phase changes the surfactants' affinity, leading to minimum curvature and nanoemulsion formation with gentle mixing. Excess aqueous phase post-nanoemulsion formation minimally affects droplet size. The order of component addition significantly impacts nanoemulsion properties, with adding water to oil producing smaller droplets than the reverse. Ternary-phase diagrams are crucial in optimizing component content. Unlike PIT, PIC doesn't rely on thermal-sensitive surfactants, offering more surfactant choices and lower-energy requirements, which is suitable for thermal-sensitive compounds. However, it demands precise control of the physicochemical properties of the components.

## 11.10 HIGH-ENERGY METHODS

High-energy methods are extensively used to formulate nanoemulsion. High mechanical energy provides strong disruptive forces, which break up large droplets into nanosized droplets and produce nanoemulsions with high kinetic energy. The disruptive forces are created by using mechanical devices such as ultrasonicators, microfluidizers, and high-pressure homogenizers [17]. By using high-energy methods, we can achieve greater control of particle size with a choice of formulation composition. High-energy methods also provide controls for stability, rheology, and

color of the emulsion [18]. In the case of food ingredients, high-energy methods of nanoemulsion formulation have the advantage of reducing the risk of spoilage and inactivation of food components without affecting food safety and nutritional and sensory attributes [19]. The high-energy methods mainly include high-pressure homogenizers, microfluidizers, and ultrasonicators. Table 11.3 describes the industrial-scale design parameters for these methods.

## 11.11 HIGH-PRESSURE HOMOGENIZATION

High-pressure valve homogenization (HPVH) is based on forcing a coarse emulsion through a narrow valve under high pressure. A coarse emulsion is typically prepared by blending oil, water, and emulsifier using a high-shear mixer. This emulsion is then fed into the inlet of the HPVH device. A pump pulls the emulsion into the homogenizer and forces it through a narrow valve. Disruptive forces such as cavitation, shear, and turbulence cause the large oil droplets in the coarse emulsion to break up and form smaller droplets [20]. The size of the droplets can be reduced by reducing the gap size of the valve, increasing the number of cycles, and increasing the pressure.

HPVH is one of the most common emulsification methods used to form emulsions and nanoemulsions in the laboratory and factory. Several studies have shown that it can be used to produce nanoemulsions. High-pressure homogenizers are used to create intensely disruptive forces that form nanoemulsions of extremely low particle size [13].

Goh et al. evaluated the effect of the number of passes through the homogenizer (1, 3, and 5) on the formation and stability of kenaf seed oil nanoemulsions [21]. The mean droplet diameter of the nanoemulsions produced depends on the homogenization conditions used. One pass was optimal for obtaining stable nanoemulsions with nanosized droplets, low polydispersity, and high charge. In contrast, additional passes were more time-consuming and costly, with a slight further reduction in droplet size. Liu et al. examined the impact of homogenization pressure on the formation of cinnamon oil nanoemulsions using HPVH [22]. They showed that particle size first decreased but then increased with increasing operating pressure. The initial decrease in size with increasing pressure can be attributed to the greater disruptive forces. In contrast, the later increase in size can be attributed to the loss of emulsifier functionality at high temperatures.

High-pressure homogenizers supply high energy and give homogeneous flow to generate the smallest particle sizes. Therefore, high-pressure homogenizers are most widely used to prepare nanoemulsions.

The coarse emulsion is then passed through a small orifice with high pressure (500–5,000 psi) (Figure 11.4). Several forces, such as intense turbulence, hydraulic shear, and cavitation, are applied together during this process, to give nanoemulsions with very small droplet sizes [23, 24].

The particle size of nanoemulsions produced by high-pressure homogenizers depends on sample composition, homogenizer type, and homogenizer operating conditions such as energy intensity, time, and temperature [25].

Increasing the intensity of homogenization decreases the size of droplets of the nanoemulsions. In some instances, such as when biopolymers are used as an emulsifier, intense homogenization can increase the particle size of the resulting

## TABLE 11.3
## Design Considerations for Performing High-Energy Methods at Industrial Scale

| High-Pressure Homogenizer | Microfluidizers | Ultrasonicators |
|---|---|---|
| **Operating Pressure:** | **Interaction Chamber Design:** | **Ultrasonic Frequency and Power:** |
| Pressure range: Industrial homogenizers operate at pressures up to 2,000 bar (200 MPa). | Microchannels: Y-shaped or Z-shaped channels where two fluid streams collide at high velocities, enhancing droplet disruption. | Frequency range: Typically between 20 and 40 kHz. Lower frequencies produce larger cavitation bubbles and more violent collapse. |
| Impact on droplet size: Higher pressures increase shear forces, leading to smaller droplets. Optimal pressure depends on formulation properties. | Uniform shear field: Consistent microchannel geometry ensures uniform processing conditions. | Power density: Higher-power inputs increase cavitation intensity, enhancing droplet disruption but also increasing heat generation. |
| **Number of Passes:** | **Operating Pressure:** | **Amplitude of Vibration:** |
| **Single vs. Multiple Passes:** Multiple passes can further reduce droplet size and narrow size distribution but increase processing time and energy consumption. | Pressure range: Typically operate between 500 and 1,700 bar (50–170 MPa). | Adjustable amplitude: Controls the intensity of cavitation; higher amplitudes lead to smaller droplet sizes. |
| **Valve Design:** | Pressure control: Precise pressure regulation is crucial for reproducibility and scaling up. | **Sonication Time:** |
| **Interaction Chamber Geometry:** The design of the homogenization valve affects flow patterns and shear rates. Common designs include radial and axial flow valves. | **Recirculation and Passes:** | Longer sonication increases exposure to cavitation but may lead to degradation of sensitive components. |
| **Flow Rate and Throughput:** Industrial homogenizers are designed for high throughput to meet production demands. Short residence times reduce processing time but may require higher pressures or multiple passes for effective size reduction. | Recirculation loop: Allows continuous processing and repeated passes through the interaction chamber to achieve desired droplet sizes. | **Probe Design:** |
| | Number of passes: Increasing passes can improve homogeneity but may affect temperature and energy consumption. | Titanium is commonly used due to its durability and inertness. The shape and size of the probe affect cavitation intensity and distribution. |
| | **Flow Rate and Scalability:** | **Scaleup Considerations:** |
| | Throughput: Designed for scalability, microfluidizers can process large volumes while maintaining nanoemulsion quality. Uniform flow ensures consistent processing across the entire batch. | Industrial Ultrasonicators often use flow-through cells to process larger volumes continuously. |

**Droplet Size and Distribution:** Controlled by shear forces generated, influenced by pressure (HPHs and microfluidizers), power input (ultrasonicators), and number of passes.

**Stability:** Smaller droplet sizes and uniform distribution enhance physical stability due to reduced gravitational separation and coalescence.

**Temperature Regulation:** Integrated cooling systems mitigate temperature rises due to high-shear forces, protecting temperature-sensitive components.

**Scalability:** Equipment must maintain nanoemulsion quality when scaling up from laboratory to industrial production.

**Material of Construction:** Compliance with regulatory standards: Materials in contact with the product must meet GMP standards and compatible with pharmaceutical ingredients.

**Energy Efficiency:** Efficient design reduces operational costs.

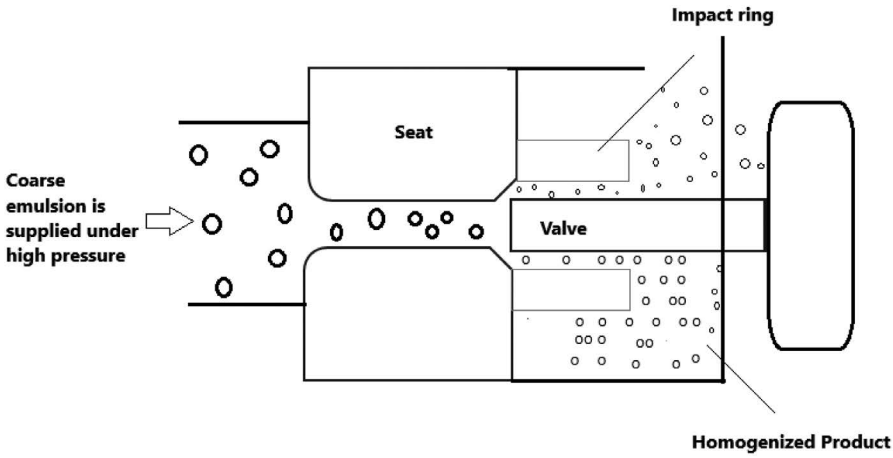


**FIGURE 11.4** Schematic diagram of High-Pressure Valve Homogenizer.

nanoemulsion. Hence, small-molecule surfactants should be used as emulsifiers in high-pressure homogenizers as they are more effective than biopolymers for producing nanoemulsions [6, 26]. High-pressure homogenization is widely used to form food, pharmaceutical, and biotechnological ingredient nanoemulsions [27, 28].

### 11.11.1 Microfluidization

Microfluidization is a mixing technology at the micro size level that uses a device called a microfluidizer. In microfluidization, fluids are forced to pass through the microchannels under high pressure (500–20,000 psi). Microchannels are generally microsized channels that allow mixing at the microsize level [29, 30]. The phases of the macroemulsion (aqueous and oil phases) are mixed together and then passed through the microfluidizer (Figure 11.5). The macroemulsion is guided through the microchannels under high pressure toward the interaction chamber, where two streams of macroemulsions strike each other at high velocity.

This collision creates forces like shearing, cavitation, and impact, which produce stable nanoemulsions [31–34].

Microfluidizers produce narrower and smaller nanoemulsion particle size distributions than homogenizers [35, 36]. Also, microfluidizers produce stable nanoemulsions at low surfactant concentrations (0.5–5%) [37]. Microfluidization methods have been used to produce food ingredient nanoemulsions [38, 39]. Microfluidization techniques produce food-grade nanoemulsions with uniform droplet size distributions and greater stabilities [40].

### 11.11.2 Ultrasonication

Ultrasonication is quickly becoming a popular method for fluid processing because it's cost-effective, requires little maintenance, has few moving parts, and boasts high-energy efficiency—about 80–90% of the power from the plug reaches the

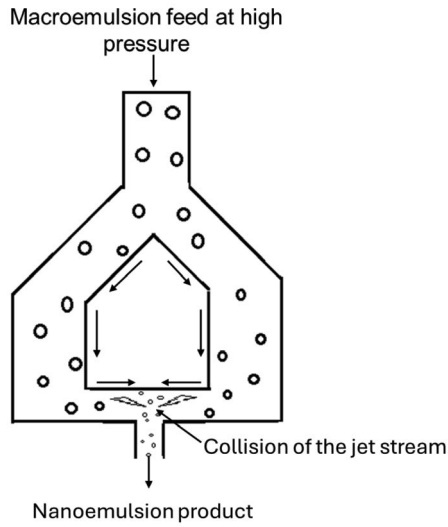


**FIGURE 11.5** Schematic diagram of a Microfluidizer.

liquid (Figure 11.6). Ultrasonicators are highly versatile machines capable of tasks like homogenization, emulsification, dispersion, reducing particle sizes, degassing, extraction, and breaking down cells, which can be an alternative to sterilization. They're especially effective at producing high-quality, uniform nanoemulsions and are increasingly used.

The technology generates ultrasonic sound waves that create alternating low- and high-pressure cycles in the liquid. This leads to the formation and collapse of tiny

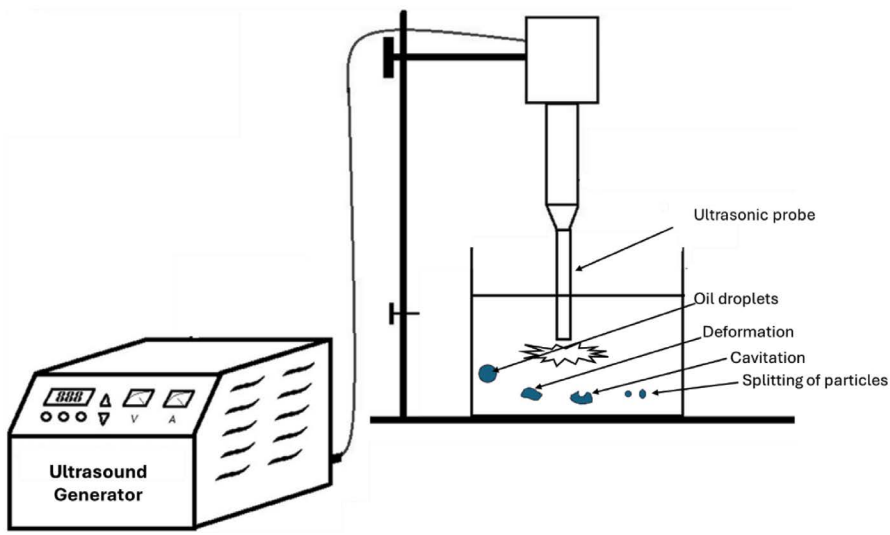


**FIGURE 11.6** Schematic diagram of an Ultrasonicator.

bubbles in a process called cavitation. Cavitation produces shear forces that mix oil and water, effectively emulsifying them.

However, scaling up this process for industrial use poses challenges. It requires a continuous flow of oil and water phases, which can be difficult to manage. Increasing the scale can also reduce production efficiency and result in a wider particle size distribution. Larger volumes need more power to maintain the same specific energy per volume as in lab-scale trials.

Ultrasonication is better than other high-energy methods in terms of operation and cleaning [32, 41]. In ultrasonic emulsifications, ultrasonic waves provide cavitation forces that break the macroemulsion to nanoemulsion. In this method, ultrasonicators are used, which consist of a probe that emits ultrasonic waves. By varying ultrasonic energy input and time, we can achieve the desired particle size and stability of the nanoemulsion. In ultrasonic emulsification, physical shear is mainly provided by the process of acoustic cavitation [42]. Cavitation is the phenomenon of the formation and growth of microbubbles. Then, the collapse of microbubbles, which is caused by the pressure fluctuations of the acoustic wave (Figure 11.6). The collapse of microbubbles causes intense turbulence that causes the formation of nanosized droplets [41, 43].

However, processing on an industrial scale requires a continuous flow of both the oil and water phases, which proves challenging. Often, a scaleup can compromise production efficiency and the resulting particle size distribution. With larger volumes, an increase in power requirement is needed to apply the same specific energy per volume as in a lab-scale trial.

## 11.12 IMPROVING NANOEMULSIONS

Various approaches have been studied to improve nanoemulsions' droplet size, content, and stability. Combining production techniques is one approach to obtaining smaller and more stable nanoemulsions.

Sarheed et al. designed a novel method combining homogenization, ultrasound, and phase-inversion techniques to produce nanoemulsions [44]. Two different production routes using these techniques were tested. Both PIT and PIC were involved in the formation of nanoemulsions. The authors found that the effect of surfactant concentration on droplet size depends on the production route used. Ultrasonication time longer than 5 minutes led to larger droplets and thus should be avoided. Combining high- and low-energy techniques can produce nanoemulsions with small droplet sizes (less than 45 nm) and long-term storage stability. Another study shows the combination of PIC and ultrasonication for producing W/O nanoemulsions [45].

## 11.13 NOVEL PRODUCTION METHODS HAVE BEEN DEVELOPED TO PRODUCE NANOEMULSIONS

Membrane emulsification is one of the novel methods. It has low-energy requirements, low-shear rate, and no temperature increase, making it suitable for processing sensitive compounds. There are two main types of membrane emulsification methods. In direct membrane emulsification, the dispersed phase is forced through a membrane into a continuous phase to form nanoemulsions. In premix membrane

emulsification, a coarse emulsion is prepared first and then passed through the membrane to form nanoemulsions.

The droplet size is controlled by membrane pore size. Premix membrane emulsification is generally better due to a higher-flow rate and the production of smaller droplets. Alliod et al. studied premix membrane emulsification using Tween 20, Span 80, and mineral oils with different viscosity but similar IFT with water [46]. Hydrophilic Shirasu porous glass (SPG) membranes were used for O/W nanoemulsion production, and hydrophobic SPG membranes were used for W/O nanoemulsion production.

Continuous-phase viscosity was found to have a more significant effect than dispersed-phase viscosity. Higher continuous-phase viscosity caused an increase in membrane pressure and led to smaller droplets. O/W nanoemulsions obtained were also much smaller than W/O nanoemulsions. Another study attempted to scaleup the premix membrane emulsification to 500 mL volume and 200 mL/min production rate [47]. The resulting pressure was found to be similar within the volume studied. A higher-flow rate caused higher resulting pressure and produced smaller droplets. Nanoemulsions around 260 nm and stable within a 9-month storage period were obtained. The authors expect the method to be scalable to larger volumes. Membrane emulsification shows good potential for producing nanoemulsions. Nevertheless, constant cleaning of the membrane is required.

## 11.14 D-PHASE EMULSIFICATION

D-phase emulsification is another novel low-energy method for the production of nanoemulsions. The main difference between this method and phase-inversion methods is that instead of forming a rigid liquid crystalline phase during the emulsification process, an isotropic D-phase is formed. This isotropic phase allows easier dispersion of the oil phase in water and leads to easier formation of nanoemulsions. Therefore, nanoemulsions with high vegetable oil content and low surfactant concentration can be produced [48]. D-phase emulsification also does not rely on strict adjustment of HLB-like phase-inversion methods; thus, a single surfactant system can be used. However, specific additives, such as alkyl polyol, may be needed to prevent the formation of a liquid crystalline phase during emulsification. Yukuyama et al. [48] optimized a D-phase emulsification process and obtained a nanoemulsion system with 40% olive oil and 2% Oleth 20. The smallest droplet size was 28 nm. The authors showed that no emulsion was formed using the same setting with PIT or PIC because no phase inversion occurred and no liquid crystalline phase was formed. They also reported that D-phase emulsification can produce similar nanoemulsions as with the HPH method under the same conditions [49]. D-phase emulsification is an interesting method for producing nanoemulsions with high oil content. However, it is less well-known, so more studies should be conducted on this method.

## 11.15 CHARACTERIZATION OF NANO/MICROEMULSIONS

Characterization of nanoemulsions and microemulsions involves a combination of analytical techniques that together provide a comprehensive understanding of their physicochemical properties [50]. Globule size and morphology influence drug release

and absorption, while rheological properties affect processing and application. IFT measurements and phase behavior studies guide the formulation process. Thermal and spectroscopic analyses reveal stability and molecular interactions, and stability studies ensure product reliability. A thorough characterization is essential for successfully developing and optimizing these colloidal systems in pharmaceutical applications.

*Particle size analysis*: Particle size and distribution significantly influence the stability, appearance, drug release, and bioavailability of nano/microemulsions.

*Dynamic light scattering (DLS)*: DLS measures the fluctuations in light scattering due to Brownian motion of particles, providing information on average particle size and polydispersity index. It is a preferred method for sizing particles in the nanometer range.

*Laser diffraction*: This technique determines particle size based on the angle and intensity of light scattered by particles, suitable for a broad size range but less sensitive for particles below 100 nm.

*Nanoparticle tracking analysis (NTA)*: NTA visualizes and tracks individual nanoparticles in real-time, providing high-resolution size distribution and concentration data.

*Morphological studies*: Understanding the morphology of nano/microemulsions helps elucidate their internal structure and stability mechanisms. Transmission electron microscopy (TEM): TEM provides high-resolution images of the internal structure of emulsions. Techniques like cryo-TEM preserve the native state of the emulsions by avoiding dehydration. Scanning electron microscopy (SEM): SEM offers surface morphology details but requires sample preparation that may alter the emulsion's structure. Freeze-fracture methods can help maintain structural integrity. Atomic force microscopy (AFM): AFM allows imaging of emulsions under near-physiological conditions, providing topographical information at the nanoscale without extensive sample preparation.

*Phase behavior analysis*: Phase behavior studies are vital for formulating stable nano/microemulsions and understanding their formation conditions. Phase diagrams: Constructing pseudo-ternary phase diagrams helps identify the concentration ranges of components where nano/microemulsions are formed. Systematic variation of oil, water, and surfactant concentrations visually represents phase boundaries.

*Visual observations*: Initial assessments of transparency, turbidity, and phase separation offer insights into the homogeneity and stability of emulsions.

*Rheological measurements*: Rheology provides information on emulsions' flow and deformation behavior, which is important for processing and application. Using rheometers, the viscosity of emulsions can be measured under varying shear rates. Microemulsions often exhibit Newtonian flow, while nanoemulsions may show shear-thinning behavior.

The oscillatory rheology technique can be used to assess viscoelastic properties by applying oscillatory stress, which reveals the structural organization and interactions within the emulsion.

The reduction of IFT by surfactants is critical for the formation and stability of nano/microemulsions.

The pendant drop method measures IFT by analyzing the shape of a droplet suspended in another fluid. It is suitable for a wide range of IFTs. Spinning drop

tensiometry is ideal for measuring ultra-low IFTs common in microemulsions, where a droplet is spun at high speed and the equilibrium shape is analyzed.

*Thermal analysis*: Thermal properties provide insights into the stability and phase transitions of emulsions.

Differential scanning calorimetry (DSC) measures heat flow associated with temperature-induced transitions, such as melting, crystallization, or phase separation, indicating thermal stability. Thermogravimetric analysis (TGA) assesses weight changes under controlled temperature conditions, which is useful for studying thermal degradation and component volatility.

*Spectroscopic methods*: Spectroscopy can reveal molecular interactions and structural details of nano/microemulsions. Nuclear magnetic resonance (NMR) spectroscopy provides information on molecular dynamics and interactions. Pulsed-field gradient NMR can measure diffusion coefficients, aiding in understanding droplet mobility. Fourier transform infrared spectroscopy (FTIR) identifies functional groups and can detect interactions between surfactants, cosurfactants, and oil or water phases.

*Zeta potential measurements*: Determines the surface charge of droplets, which affects stability due to electrostatic repulsion or attraction. A higher absolute zeta potential generally indicates better stability.

*Stability studies*: Assessing the stability of nano/microemulsions is crucial for their practical application. This includes accelerated stability testing by subjecting emulsions to stress conditions like centrifugation, freeze-thaw cycles, or elevated temperatures to predict long-term stability and identify possible instabilities such as creaming, coalescence, or phase separation. Also, the chemical integrity of the components over time should be monitored, including oxidation of oils or degradation of surfactants and active pharmaceutical ingredients.

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# 12 Nanocoated Medical Devices

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## 12.1 INTRODUCTION

Medical devices (MDs) have been in clinical use for many years, with a noteworthy rise in their application and the integration of implants over the past few decades. This increase is largely attributable to the growing demand for advanced therapeutic solutions, diagnostic tools, and patient-specific care. The recent surge in chronic diseases, aging populations, and the requirement for minimally invasive procedures has further pushed this trend. The MD industry is projected to grow remarkably, reaching an estimated valuation of USD 886.80 billion by the end of 2032, up from approximately USD 518 billion in the current scenario [1]. A MD is defined as any instrument, apparatus, appliance, material, or other product intended by the manufacturer for use in humans for the following purposes: (1) Diagnosis, prevention, monitoring, treatment, or alleviation of a disease, injury, or disability, (2) Investigation, replacement, or modification of anatomy or a physiological process; (3) Control of conception. Such devices do not achieve their primary intended effect through pharmacological, immunological, or metabolic means, although they may be assisted by such methods in their function [2, 3]. Advancements in nanotechnology have revolutionized the field, enabling the development of next-generation nanocoated MDs. These nanocoatings enhance the therapeutics outcomes by providing reduced drug dose, enhanced cellular uptake, longer tissue residency, prolonged drug release. This minimizes the need for repeated surgical interventions, thus reducing patient risk. Additionally, the enhanced biocompatibility, durability improves their interaction with biological compartments, reduces the risks of infection or rejection leading patient compliant therapy. This chapter comprehensively summarizes the applications of nanocoated implantable MDs, fabrication techniques, biocompatibility, safety concerns, recent advances, and challenges.

## 12.2 TYPES OF NANOCOATINGS

Nanocoating involves covering a surface or nanometric structure with a thin film (thickness—100–1000 nm) to add new functions. Comparing nanocoatings to traditional coating materials reveals that these have better adhesion, increased flexibility, and improved resistance to wear and tears. These coatings are of different types as described below.

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### 12.2.1 Polymeric Nanoparticles

Different degrees of success are achieved when using polymer materials as coatings with or without drug to it. For devices like stents and catheters, polymer coatings can improve the mechanical characteristics of the product by allowing for strength and flexibility [4]. Since they are currently being utilized for implants or other MDs, poly(ethylene) (PE), polyurethanes (PUR), poly(glycolide) (PGA), and polylactides (PLA) have all been assessed as potential MD coating materials [5]. Owing to their effectively demonstrated hemocompatibility, such stents have been used since the early 1990s for the treatment of esophagorespiratory fistulas, malignant biliary, esophageal or gastroduodenal obstruction, arterial diseases, and irresectable esophageal carcinoma. The primary issue with non-biodegradable polymer layers is their degradation products, which occur when they interact with the biological fluids and have the potential to cause inflammation and the subsequent formation of thrombosis. Because polymers are prone to breaking, there is a risk that material fragments will be released into the bloodstream and cause some of the damaged vessels' narrowed areas to close [6]. Poly(L-lactic acid), poly(D,L-lactide), polycaprolactone (PCL), and poly(lactide-co-glycolide) are the main components of biodegradable polymers [7]. The final polymer decomposes into carbon dioxide and water, while the initial three polymers yield lactic acid. Use of these polymers in nanoparticulate system for coating on drug-eluting stents (DESs) may help prevent undesirable outcomes such as in-stent restenosis (ISR), late stent thrombosis, and hypersensitivity reactions because they degrade once their purpose is fulfilled [8].

### 12.2.2 Metal-Based Nanocoatings

Metallic nanoparticle-based thin films are applied to MD surfaces to improve their functional characteristics. These coatings often have antimicrobial properties, enhanced biocompatibility, increased wear and corrosion resistance, and make it easier for therapeutic substances to be released under controlled conditions. Because titanium is compatible with human tissue, it can be effectively integrated into dental, orthopedic, and cardiovascular devices, among other applications. Its use in applications where long-term stability and durability are crucial is further enhanced by its capacity to promote osseointegration in orthopedic implants. All things considered, titanium is a favored material in the medical industry due to its blend of mechanical strength and biocompatibility [9]. A new range of stents coated with titanium oxynitride. It has been demonstrated that the vapor-deposited $TiO_xN_y$ and TITAX-AMI products are safe and effective in lowering ISR and myocardial infarction risk [10]. Titanium and its oxides lower the chance of inflammation and improve implants' resistance to corrosion. When it comes to stents, titanium oxide lessens platelet aggregation and fibrinogen coagulation by preventing the electrons from fibrinogen from moving to the surface.

### 12.2.3 Ceramic Nanocoatings

These coatings, which are commonly derived from bioceramics like hydroxyapatite (HA) or alumina, are intended to decrease the risk of infection, enhance durability,

and enhance the device's integration with biological tissues. As a result, they are well suited for a variety of applications, such as implants, surgical instruments, and dental devices. They are much more resistant to oxidation, corrosion, and wear than metals, specifically in high-temperature applications. They also offer superior electrical and thermal insulation qualities. In general, compatibility with the human body—that is, not causing a negative reaction in the body and not being destroyed under the influence of bodily fluids—should be considered when selecting a material and manufacturing technology for an implant. This includes being non-toxic, non-carcinogenic, non-allergic, non-thrombogenic, etc. For several decades, ceramic coatings have been used in orthopedics by manufacturers. Titanium nitride (TiN) coatings of various hips, knees, and other devices have been shown. CoCr alloy is still the preferred metal for these bearing surfaces because of its high hardness and wear resistance. In addition to the benefits listed above, ceramic coatings have the potential to improve the lubrication between the metal and polyethylene components by increasing hardness, decreasing surface roughness, increasing wettability, and lowering the coefficient of friction [11].

## 12.3 FABRICATION TECHNIQUES

Following are a few techniques used to coat or deposit the nanomaterials on MDs.

### 12.3.1 Physical Vapor Deposition (PVD)

One approach that is often used to cast thin films onto substrates is physical vapor deposition, or PVD. The target, or material to be deposited, is converted into atomic particles by a thermal, physical collision process in PVD processes. The atomized particles are then directed toward the substrates in a low-pressure, vacuum environment or gaseous plasma, where they condense to form a physical coating [12]. Deposition can either be accompanied by the sputtering process or by the evaporation procedure. A typical PVD process is depicted in Figure 12.1.

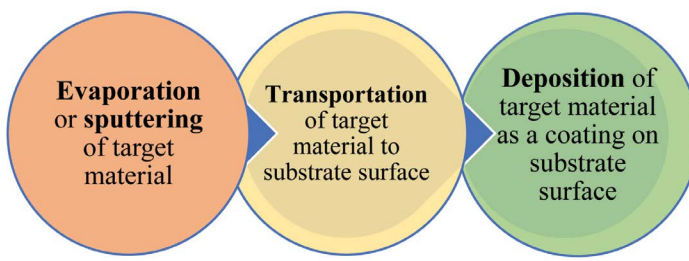


**FIGURE 12.1** Both PVD sputtering and PVD evaporation are deposition techniques where atomic-sized particles are deposited onto a substrate. In sputtering, a magnetron accelerates ionic gas to cause the target to release particles, which are then deposited. In evaporation, material is heated by an electron beam to release particles, creating a plasma, both of which intensifies in the chamber and deposits particles onto the substrate in layers for strong adhesion.

PVD sputtering procedures involve the placement of a magnetron close to the target. Accelerated introduction of the ionic gas into the vacuum chamber causes the target to explode, releasing atomic-sized particles that will be deposited and forcefully projected onto the substrate [13].

In PVD evaporation, the material to be evaporated is contained in an evaporation source on the target, which serves as a cathode. The particles, which are also atomic in size, evaporate when heated by an electron beam. A plasma that travels through the deposition chamber and becomes more intense in the center of the reactor chamber is created when the released particles collide with the gas molecules that are added to the reactor to accelerate the particles. Particles will be applied to the substrate in layers that are gradually compressed, ensuring that the film adheres to the substrate well.

In contrast to commonly used amorphous carbon films, innovative fullerene-like, carbon-based nanostructured coatings, such as fullerene-like carbon nitride (FL-CNx) developed using the magnetron sputtering technique, possess low water absorption due to the absence of dangling bonds. For magnetic storage devices, FL-CNx coatings are therefore appropriate protective overcoats. PVD does have some restrictions, though. For instance, it might not be appropriate for the deposit of some materials, like organic compounds or polymers. Moreover, high vacuum conditions are important, which hinder its scalability for industrial production. Despite the drawbacks, PVD is widely utilized in a number of sectors, including aerospace, electronics, and optics, because it is helpful in creating coatings with particular characteristics, like resistance to corrosion or wear.

### 12.3.2 Chemical Vapor Deposition (CVD)

Using the chemical vapor deposition (CVD) method, vapor or gaseous material is separated by chemical reactions in the immediate area of a heated substrate surface, and the coating is then deposited on the heated substrate surface. Materials that are metallic or ceramic are typically used as substrates. The substrate's temperature plays a vital role in determining how well the coating adheres to it [14]. To guarantee the desired thin-film properties, the reaction conditions, including pressure, temperature, and rate of gas flow, are thoroughly regulated. Figure 12.2 illustrates a CVD process.

Among the materials that CVD can deposit are metals, semiconductors, and insulators. It is a flexible process that can yield films of excellent quality with exact control over composition, thickness, and crystal structure. In nanofabrication, CVD is widely used to create advanced materials such as optoelectronics and microelectronics [14]. A DES for CVD was created by Zhi and Mao to treat coronary artery disease with atorvastatin and a sustained zero-order release of the antiproliferative medication sirolimus. The atorvastatin and sirolimus showed zero-order release for 30 days with a molar ratio of 0.78 for the monomer to cross-linker. The drug release rate was linearly correlated with the dose of the encapsulated drug, offering a straightforward but effective method of controlling drug release. At as low as 5 $\mu g/cm^2$, it inhibited the viability and proliferation of HCASMC (Human Coronary Artery Cells Culture and Cell Activity Study). Furthermore, by applying nanocoating to the substrate, there was significant reduction observed in platelet adhesion without adversely affecting red blood cells [15].

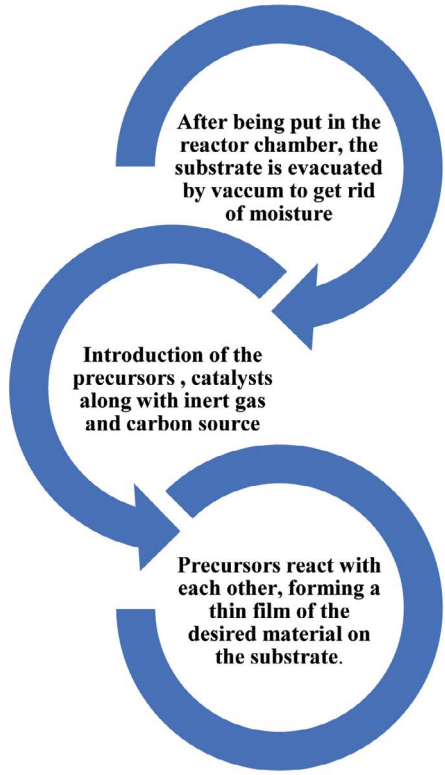


**FIGURE 12.2** Schematic representation of the deposition process involving substrate evacuation in a vacuum chamber followed by the introduction of precursors, catalysts, inert gas, and carbon sources, resulting in the formation of a thin film on the substrate surface.

### 12.3.3 Sol-Gel Synthesis

This process comprises of mixing of metal alkoxides or other inorganic chemicals with a solvent, to create a precursor solution. Following that, hydrolysis and condensation of the mixture is performed, yielding a gel that will further undergo annealing or calcining to form a solid state. The gel can undergo additional further processing such as sintering or calcination to obtain the required nanomaterials with precise size, composition, and shape [16]. Figure 12.3 depicts the sol-gel synthesis process. This process is very helpful in creating nanocoatings on a variety of materials, including composites, glasses, and ceramics. Numerous factors such as, temperature, pH, nature of the catalyst, the starting material used to make sol has a great impact on the coating density and hydrolysis rate, developed using the sol-gel process. Unlike other nanofabrication techniques, sol-gel synthesis is different in that, while maintaining high purity and low processing temperature, it can create a broad range of forms and architectures [17].

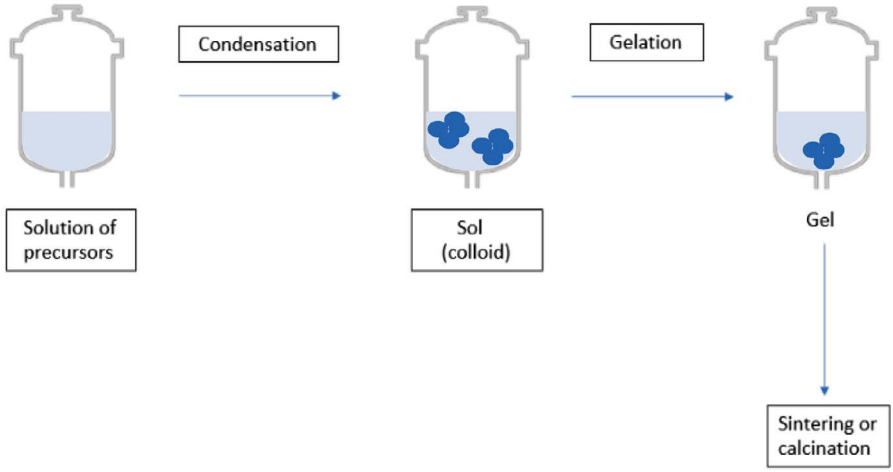


**FIGURE 12.3** Schematic illustration of the sol-gel process, where metal alkoxides or inorganic compounds are mixed with a solvent to form a precursor solution, followed by hydrolysis and condensation to produce a gel. The gel is then annealed or calcined, with optional further processing such as sintering, to obtain nanomaterials.

### 12.3.4 Electrospinning

The flexible method of electrospinning is extensively employed in biomedical applications to fabricate scaffolds and nanofibers (NFs) for drug release and tissue engineering [18]. The most basic setup for this method consists of a pump, a high-voltage power source, a collector, and a solution inside a reservoir, usually a syringe, that is tipped with a blunt needle (at least for needle-based electrospinning).

When a certain voltage is applied to generate an electric field between the tip of the needle and collector, starts off the spinning process. Charges build up at the liquid's surface as the pump maintains a steady flow of the solution. The liquid meniscus soon reaches a point where surface tension is greater than electrostatic repulsion, which causes the liquid meniscus to change form into the conically shaped structure named as a Taylor cone. At the needle's tip, the flow transitions from dripping to a charged cone-shaped structure. The charged liquid jet ejects toward the collector once the Taylor cone forms as depicted in Figure 12.4.

Due to the excellent mechanical qualities and large specific surface areas of the fibrous structures created by this process, electrospinning has become a popular method for creating nanoscale fibers for tissue engineering [19]. Additionally, NFs provide a favorable microenvironment for cell adhesion and expansion by imitating the structure of the extracellular matrix (ECM) found in nature [20]. To deliver rosuvastatin (5 μg/mm$^2$), Lee et al. developed a covered DES using electrospinning, based on poly(D,L)-lactide-co-glycolide poly(lactic-co-glycolic acid) (PLGA) NFs. In a rabbit model, a marked decrease in platelet adhesion and an improvement in *in vivo* re-endothelialization were reported [21].

In the biomedical industry, electrospinning systems must be highly precise, dependable, and compatible with materials in order to generate fibers with consistent

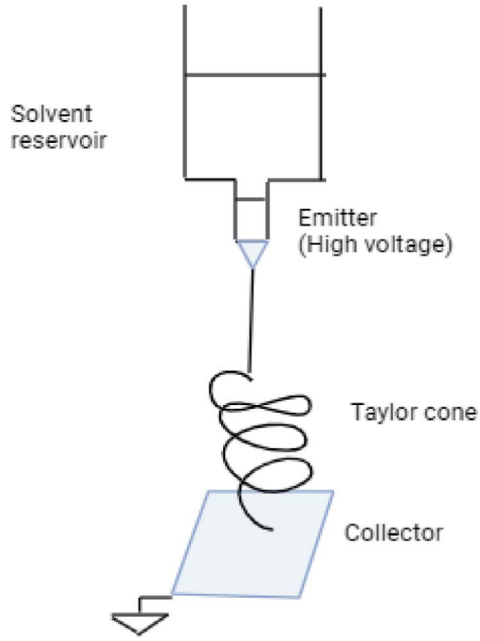


**FIGURE 12.4** Schematic overview of the electrospinning process, where a high voltage generates an electric field that induces the formation of a Taylor cone and propels a charged liquid jet toward a collector, resulting in the formation of nanoscale fibers with high mechanical strength.

characteristics such as high surface area and controlled diameters, which are essential for drug release, nutrient diffusion, and cell adhesion [22].

The fundamental unit for industrial production is the Nanospider™ (Figure 12.5) NS 8S1600U. It is a modified electrospinning technique based on a rotating spinning electrode and is characterized by being scalable to up to four units and designed for 24-hour, 7-day operation. The polymer, substrate, process, and fiber diameter all affect throughput. Additionally, the Nanospider enables the simultaneous production of multiple Taylor cones, making this technique extremely productive even though it necessitates the use of a higher voltage [23].

Emulsion electrospinning based on Nanospider® was used to create chitosan–PVA/PLLA NFs with *Hypericum perforatum* L. extract to promote effective wound healing. Two different concentrations of *H. perforatum* L. (HP) were added to the electrospun PLLA/PVA/CS fiber mats (2.5% and 5.0%). Since the electrospun PLLA/PVA/CS fiber mats' total porosity, wettability, water vapor transmission rate (WVTR), and swelling characteristics were comparable to those observed in the skin's ECM, the results showed that they were perfect for use as wound dressings [24].

### 12.3.5 Spray Coating

The process of spray coating involves passing a target material through a nozzle and impacting it onto a substrate surface to produce a coating. One of the most popular techniques for applying spray coating is thermal spraying. To achieve a consistent

**FIGURE 12.5** Schematic representation of the electrospinning setup comprising a rotating collector, spinning electrode, and high-voltage power source. The polymer solution is fed through a nozzle, where the electric field generates nanofibers that are deposited as a fine, homogeneous fiber mat on a grounded surface [23].

deposit of a uniform drug release layer(s), the polymer and drug solutions are first ultrasonically atomized by injecting them into the atomization chamber through electrohydrodynamic jetting. This is followed by an air brush spray coating of the mixture onto the substrate. The ultimate characteristics of the sprayed coating are determined by variables like spray velocity, distance between substrates and spray gun, powder feed rate, etc. Using thermal spraying, supporting materials with bigger surface areas can be coated at higher rates of deposition than with other traditional techniques like CVD and PVD. The fact that this is the simplest method for

increasing the production of coated MDs by producing large quantities of consistently coated devices may contribute to its popularity. For the purpose of researching its potential as a DES release platform, a PLGA, ReoPro (abciximab), and PVA emulsion were sprayed onto 316L SS disks. According to this study, there was a 56% burst release of ReoPro during the first four days when the weight percentage of PVA was increased. A burst release of the drug is attributed to its presence at the stent-vascular environment boundary layer, which is then followed by a slower release of the drug to facilitate long-term therapeutic effects [25]. It is most widely used for coating drug-eluting devices.

SonoTek technology (Figure 12.6) is considered perfect for applications needing scalable solutions and high-quality thin films because it gives exact control over

**FIGURE 12.6** Schematic depiction of SonoTek ultrasonic spray coating technology, utilizing high-frequency ultrasonic waves to atomize liquids into fine droplets. The system includes an ultrasonic transducer, a generator to power the transducer, and a nozzle that directs the atomized mist onto the substrate for precise coating applications [26].

droplet size, spray angle, and coating thickness. One characteristic that sets SonoTek systems apart is the use of ultrasonic atomization (vibration) to break liquid coatings (like polymers, metals, or inks) into fine droplets in order to produce extremely uniform and controlled coatings on a variety of surfaces.

Regarding the type of material to be used, it is versatile; a broad variety of materials, such as nanomaterials, organic and inorganic solutions, and biocompatible coatings, can be employed. Ultrasonic coating for nanoparticles has distinct advantages. Nanoparticles have a tendency to cluster together and separate from the liquid suspension when dispensed using traditional mixing equipment and pumps. Additionally, the particles' uniformity can be restricted, and the quantity of nanomaterial required is excessive and expensive. SonoTek ultrasonic spray nozzles apply a very thin layer of nanoparticles to substrates of various sizes, shapes, and forms in a uniform and precise manner. The micronic droplets that are continuously produced by the ultrasonic spray nozzle contain the same concentration of nanoparticles. With a transfer coefficient greater than that of traditional coating technologies, the liquid evaporates by the time it reaches the target, gently positioning the nanoparticles [27].

In a study, an endothelium-mimicking nanomatrix was added to the LAAC device membrane to deliver nitric oxide (NO) to improve endothelialization, with the aim of potentially reducing the duration of the anticoagulation therapy to prevent embolic stroke due to stroke. The polyester membranes of LAAC (left atrial appendage closure devices) (Watchman, Boston Scientific) were coated with the nanomatrix coating using an ultrasonic spray coating machine (Exactacoat, Sonotek Corporation, NY). The LAAC device membrane was fully coated using an impact nozzle and an overlapping back-and-forth pattern (flow rate: 0.2 mL/min; speed: 50 mm/s; shaping air: 1.5 psi). A further layer was applied once each layer had completely dried (dwell time, 15 s). The device provided a sustained release of NO *in vitro* for up to one month. Furthermore, *in vitro*, the nanomatrix coating decreased platelet adherence and increased endothelial cell (EC) proliferation in comparison to the uncoated device membranes [28].

## 12.4 APPLICATIONS OF NANOCOATED MEDICAL DEVICES

This portion offers a comprehensive review of the nanocoated MDs, particularly concentrating on devices designed for various applications, including the arterial disease, dental, orthopedics, neuronal, retinal and cochlear, and miscellaneous implants.

### 12.4.1 Implants for Arterial Diseases

Implants principally utilized for the treatment of arterial diseases, including cardiovascular and peripheral artery diseases, are predominantly drug-eluting devices. These comprise DESs and drug-eluting balloons (DEBs), coated with drug and polymers, coatings that are applied layer by layer or entrapment of drug in polymeric matrix/lipidic core or polymer-lipid hybrid fabricated to release anti-inflammatory and anti-proliferative drugs directly into the arterial wall to provide consistent and prolonged drug release to prevent restenosis. DESs are typically, metallic stents

which are coated with drug-loaded polymers, serving as a local drug reservoir. DEBs offer a non-permanent alternative to stents by treating narrowed arteries without leaving an implant behind [29]. The coatings are primarily applied to drug-eluting devices using widely utilized spray coating techniques, where drug and polymer layers are applied sequentially. In the process of coating micro- or nanoparticles onto stents, these particulate systems are dispersed in organic solvents and applied under suitable and specific process parameters. Consistent and prolonged drug release is offered by this technique, by assuring maximum drug loading on the stent and generating a uniform coating in a short amount of time. Because of its property of decreasing neointimal hyperplasia in the stented artery, this prolonged release of the active assists in the prevention of ISR [30]. Strong mechanical integrity must exist in the coating employed on these drug-eluting devices in order to assure its durability under physiological conditions. In presence of the hemodynamic forces that are seen in the diseased vasculature, such as the increased blood flow rates seen in PAD (about 172 mL/min) and CAD (about 35 mL/min), it is crucial that the coating remains attached and does not delaminate [31–35]. To have consistent drug release and normal device function over the duration of its lifespan, the coating should also be able to put up with mechanical stressors that are often present in the arterial environment, such as pulsatile flow, shear pressures, and cyclic deformation. Drug-eluting devices that coated with biodegradable poly (lactic-co-glycolic acid) (PLGA) polymeric nanoparticles and antiproliferative cytostatic drugs such as sirolimus offer a number of benefits over the traditional drug-coated devices. These benefits consist of reduced drug dosage, improved and enhanced cellular uptake due to the nanosized particles, and longer tissue residency time, resulting in a controlled and delayed release of the therapeutic substance at the restenosis site. This consistent and prolonged drug release profile not only enhances endothelialization but also prevents the migration and proliferation of vascular smooth muscle cells (VSMCs), which are major causes of restenosis. By enhancing localized therapeutic rewards and reducing systemic drug exposure, the use of such advanced coating system improves clinical outcomes. The drug release profile of such particulate-coated stents is biphasic: An initial burst release from unentrapped drug, which is therapeutically beneficial as ISR begins immediately after stent implantation, succeeded by a sustained-release phase that aligns with the disease's pathophysiology, typically lasting from 3 months to 1 year [36–38]. In addition to DEBs, devices like SirPlux Duo utilize functional nanoparticles of SRL and PTX for the treatment of PAD. MagicTouch incorporates phospholipidic nanocarriers of sirolimus for CAD. Selution SLR™ employs MicroReservoir technology combined with PLGA and Cell Adherent Technology (CAT™) to enable extended sirolimus release for up to 90 days. Orchestra BioMed's Virtue SAB integrates sirolimus-loaded polymeric nanoparticles for the treatment of PAD [39–42].

### 12.4.2 Dental Implants

Implants with nanocoatings have been extensively used due to their ability to significantly enhance the overall biocompatibility with surrounding tissues. These nanocoatings improve the surface interactions at the cellular level, leading to a higher degree of osseointegration and more successful integration with bone compared

to implants lacking such surface modifications. Dental implants play a crucial role in supporting various dental prosthetics, including crowns, bridges, dentures, and anchorage systems for orthodontic tooth movement. These implants are widely used for the replacement of missing teeth, ranging from single-tooth replacements to full arch restorations, whether for partial or complete edentulism. Titanium (Ti) alloys are especially favored in the fabrication of metallic implants due to their superior mechanical strength and chemical stability, making them ideal for long-term use in dental applications. However, traditional polished titanium implants face challenges such as suboptimal cell adhesion, limited antimicrobial activity, and insufficient bone-bonding capabilities during clinical use. To enhance osseointegration—the direct structural and operational relation between the surface of the implant and surrounding bone—modifications to the implant surface are critical. This process can be speeded by designing implants with surfaces that possess micro- and nanoscale roughness, also known as nano/microtextured surfaces. These textured surfaces offer better integration and stability of the implant within the bone, which also enhance contact with biological tissues. The surface alteration of titanium implants is improved by the application of micro/nanostructured $TiO_2$/ZnO coatings, which have the several benefits of simplicity, stability, and ease of manufacture. They are mainly promising for comprehensive use because of their scalable production process, which improves the surface properties of titanium implants to enhance biocompatibility and encourage improved osseointegration [43]. It has been demonstrated that magnesium acetate-based nanotechnology-developed coatings encourage the adhesion, growth, and osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs). These findings imply that the presence of $Mg^+$ ion-containing nanoporous titanium coatings, which are crucial for biological processes, might encourage quicker osseointegration—the integration of the implant with the surrounding bone tissue [44]. Also, implants with nanoscale surfaces improve osseointegration by extending the region of interaction with live cells. An implant is further stabilized by improving and enhancing its surface wettability, which promotes osteoblast development and bone mineralization. The surfaces of titanium alloys are modified by processes like electrochemical oxidation, which generates nanoscale structures like tubes and pores. A popular technique called micro-arc oxidation (MAO) incorporates regulated duty cycles and dwell periods in an $Li_3B_2O_4$ solution to design nanoscale holes (10–300 nm) and micro-sized slots (3–7 μm). These alterations quicken osseointegration and improve cell adhesion [45]. Integration of nanohydroxyapatite (nano-HA) particles, which have a diameter of 26 nm, was believed to be a possible way to improve filler's ability to seal. Owing to their nanoscale size, HA particles were capable of nicely penetrating the micro-gaps and strengthening the relationship between the filler material and the substrate [46]. Studies have been performed to evaluate the *in vivo* stability of coatings on titanium implants made of HA nanoparticles. The outcomes showed that the released nanoparticles are effectively removed from the systemic circulation by the body's natural clearance processes. This recommend that there is little chance of nanocoating getting accumulated in vital organs, which reduces the health dangers connected to prolonged exposure. The study emphasizes how crucial it is for HA nanoparticles to be both biocompatible and biodegradable in order to safely integrate with surrounding tissues and to

ensure that any materials that are leached are quickly cleared from the body [47]. By improving the contact between the implant and the surrounding biological tissue, the osseointegration process is facilitated by the application of nanostructured coatings on titanium surfaces. By increasing an implant's mechanical stability and biocompatibility, these nanocoatings can provide a more effective and long-lasting association with the host tissue. It is remarkable that hybrid coatings made of bioglass and cerium oxide (ceria) have several applicational advantages, such as improved cell adhesion, antibacterial activity, and super hydrophilic qualities. These characteristics eventually aid in the long-term stability and effectiveness of implant devices by minimizing the colonization of germs and promoting cell growth. These coatings may also be tailored to regulate surface chemistry and topography, which enhances their efficacy in a range of therapeutic applications [48].

### 12.4.3 Orthopedic Implants

Implants are frequently used in orthopedics to stabilize fractures using bone screws and plates and to replace joints, such as hip and knee prostheses. Made from biocompatible materials like titanium alloys and stainless steel, these implants ensure mechanical strength and corrosion resistance. They are designed to mimic natural joint movement and provide rigid support for healing. Advanced surface treatments improve osseointegration and reduce infection risk, enhancing long-term functionality and patient outcomes. Cementless knee joint prostheses demonstrate a loosening rate of up to 28% within 4–10 years post-implantation, in addition a 2–5% infection rate is linked with orthopedic implants, including fracture fixation devices and joint prostheses. In the United States, total hip and knee arthroplasties account for over one million procedures annually. Despite the relatively minimal risk of infection or failure, the large patient population with orthopedic implants leads to significant patient suffering and healthcare burdens [49]. Nanocoatings, including pH-responsive, temperature-responsive, light-responsive, and piezo-responsive coatings, are utilized to enhance osseointegration as well as to exhibit antibacterial and antifungal properties. These specialized coatings are engineered to respond dynamically to environmental stimuli, which can facilitate localized drug delivery, promote cellular adhesion, and trigger antimicrobial actions [50]. A nanocoating developed using trimethylsilane (TMS) effectively inhibits formation of biofilm by *Staphylococcus aureus* and *Staphylococcus epidermidis*, while enhancing bone conductivity. Due to their thick, cross-linked structure, these dual-functional coatings support osteoblast activity, enable high adherence to implant surfaces, and offer abrasion resistance. They also provide long-lasting functionality. In the end, they enhance bone integration and lower the possibility of infections linked to devices in orthopedic applications [49]. Orthopedic implants may benefit from the developing field of antimicrobial nanosurface technology. By adding nanoscale characteristics that interact physico-biochemically with microbes, this method improves the antibacterial qualities of implant materials. The production of biofilms and efficient inhibition of microbial adhesion are two important aspects of implant-associated infections that can be prevented by these interactions. These coatings compromise the structural integrity of bacterial cell walls by modifying the surface topography at the nanoscale

with different forms, which lowers microbial viability. To further reduce the danger of infection, the customized nanostructures can release antimicrobial compounds over time, resulting in a confined environment. The lifetime and success rates of orthopedic implants might be greatly increased by such developments in nanosurface technology, which would also lower the frequency of post-surgical problems and the necessity for revision procedures [51]. Although polyetheretherketone (PEEK) is a strong orthopedic material, treating bone infections with it is not possible due to its bioinert nature. In order to improve biocompatibility, bone regeneration, and antibacterial qualities, a 2D nanocoating comprising graphene oxide (GO), polydopamine (pDA), and oligopeptide was applied on porous sulfonated PEEK (SPEEK). In animal models, the coated PEEK significantly improved osseointegration and bone healing, offering a new therapeutic approach for bone infection management [52].

### 12.4.4 Neuronal Implants

In cases of neurological diseases, the treatment window is often limited, leading to significant side effects and toxicity due to non-targeted drug delivery. Systemic administration requires higher doses because of the blood-brain barrier's restrictive nature, resulting in increased peripheral side effects. Thus, targeted drug delivery is preferred, though it involves penetrating the nervous system. The goal of many neural therapies is to prevent recurrence, often through long-acting drugs administered at the specific site. Using biodegradable polymers as carriers for drug delivery has gained popularity in recent years, particularly for managing neurological disorders [53–55]. Polymeric micro- and nanoparticles for site-specific delivery have shown promise in research, although challenges like burst release and expulsion due to high interstitial pressure remain. To address these, compact forms like discs, wafers, and fibers have emerged, offering a higher surface-area-to-volume ratio, improved implantability, and controlled drug release [53, 54, 56]. An example is the Gliadel® wafer, introduced in 1980 for glioma treatment. It consists of carmustine-loaded, polyanhydride-based microparticles (1–20 μm) that are compressed into 14-mm diameter wafers. Up to eight wafers can be placed in the surgical cavity after tumor resection, aiming to prevent recurrence. This approach has been shown to extend patients' survival by approximately two months compared to placebo, owing to the sustained release of the drug over about 230 days. Biodegradable polymer implants are thus increasingly recognized for their clinical efficacy [54]. Sustained drug release is essential, but the implant's geometry also plays a critical role in treatment outcomes, as glioma recurrence is often observed within 2 cm of the resection site. Ranganath and Wang [56] explored this by developing paclitaxel-loaded PLGA implants in various shapes using electrospinning, which provided sustained drug release over 80 days. Sub-microfiber architectures, in particular, enabled a faster release profile, highlighting the importance of design in therapeutic applications.

### 12.4.5 Retinal and Cochlear Implants

Cochlear and retinal implants have become widely adopted for enhancing hearing and vision, respectively.

Cochlear Implants: A cochlear implant system consists of (i) an external device comprising a microphone, processor, and transmitter that captures and encodes sound; (ii) a receiver-stimulator that decodes and transmits the signal; and (iii) an implanted microelectrode array positioned in the cochlea, which stimulates the auditory nerve pathways. These devices not only transmit signals but also convert audio signals into electrical impulses to facilitate hearing. Recent advancements include nano-topological modifications, polymeric nanocarriers, and neurotrophin coatings to enhance neural regeneration[57]; additionally, innovations like polydopamine coatings, as demonstrated by Kang et al. [58] improve cell adhesion and biocompatibility, while expanding the use of implants to drug and gene delivery for conditions like vertigo and sensorineural hearing loss [59]. Thus, cochlear implants have evolved beyond hearing aids into multifunctional therapeutic tools.

Retinal Implants: Vision-enhancing prostheses are categorized into two types based on implantation site: (i) retinal prostheses, which stimulate the retina and optic nerve to counteract vision loss from conditions like retinitis pigmentosa and macular degeneration, and (ii) retino-cortical prostheses, designed to stimulate the central nervous system for vision improvement in cases of optic nerve damage or CNS-related vision loss [60]. Advances include 3D microelectrode arrays developed using silicon deposition and flexible polyimide-Parylene-C coatings [61]. Additionally, diamond-based retinal implants, such as those studied by Hadjinicolaou et al., use nanocrystalline diamond arrays coated with a monolithic diamond layer. These implants demonstrate enhanced electrochemical stability and can effectively stimulate retinal ganglion cells, making diamond-based implants a promising future option for vision restoration [62].

### 12.4.6 Miscellaneous Implants

Other types of nanocoated implants include ocular lenses, vaginal implants, and wound healing implants. To enhance drug permeation through the cornea and extend the release profile for glaucoma treatment in elderly patients, where adherence to traditional eye drops is often poor, chitosan-based nanocoatings have been developed for ocular contact lenses. The process involved atomizing the coatings to form particulate structures, which integrated effectively with chitosan, creating a stable coating layer. These coatings showed good biocompatibility with the eye and encapsulated therapeutic molecules with an efficiency of over 88%. The coatings produced either triphasic or biphasic drug release patterns, depending on the specific formulation. Enhancing drug penetration through the cornea while maintaining corneal tolerability, this technique presents a feasible way to improve patient compliance and therapeutic results in the therapy of glaucoma. Furthermore, it is reported that the novel nanocoating method may reduce the frequency of doses, improving its suitability for long-term usage in older populations [63]. PLGA-based coatings, cyclodextrin, hydrogel contact lenses with a ring loaded with ethylcellulose nanoparticles, Bri@LDH/Thermogel, and lipid nanocarrier-coated contact lenses are some more examples. These innovative formulations are meant to improve drug release over time and enhance corneal permeability [64]. Vaginal rings having polymeric nanoparticles coated on them have been fabricated to facilitate for medium- to long-term

medication administration straight to the female reproductive system and genitalia. By improving localized drug retention in vaginal tissues, these novel devices provide effective drug delivery over the mucosal barrier and deeper into the tissue. By making use of a targeted method, it is possible to sustain therapeutic agent levels that are effective for more extended than the normal turnover and to shed epithelial cells and vaginal mucus. These rings, which provide continuous delivery, can decrease the frequency of medication administration, improve patient adherence, and improve therapeutic efficacy, especially for long-term illnesses or preventive applications like hormone therapy and HIV prophylaxis [65, 66].

Furthermore, because of their noteworthy mechanical qualities and capacity for shape memory, polymeric nanoparticles show great confidence in applications related to wound healing. Their potent antioxidant properties aid in reducing oxidative stress in the wound environment, and their high biocompatibility makes interaction with biological tissues possible. These nanoparticles also encourage greater cell proliferation, which is essential for efficient tissue regeneration. Polymeric nanoparticulates have the capability to enhance wound healing results by decreasing scarring and promoting quicker healing processes through the application of these characteristics. This makes them a useful tool in tissue engineering and regenerative medicine [67, 68]. Micro- and nanotechnological advancements are primarily applied in cardiac implants, brain and retinal implants, and bone and dental implants. However, other emerging sectors also hold promise and should not be overlooked. Functional nanocarriers are known to enhance the sensitivity and specificity of diagnostics. In this context, Deng et al. developed a highly sensitive sensor for nitrous oxide measurement, utilizing a carbon electrode coated with a nafion/multi-walled carbon nanotube (CNT)-gold nanoparticle composite. This sensor demonstrated a detection limit as low as 19 nM and was biocompatible with various tissues, including the liver, kidneys, heart, and spleen, when tested in mice [69]. These promising results highlight the potential for further research in developing advanced nano sensors for medical applications.

## 12.5 BIOCOMPATIBILITY AND SAFETY CONSIDERATIONS

Although these nanocoated MDs demonstrate promising applications, they may also produce unintended effects. The following sections brief these potential outcomes and describe the tests used to confirm biocompatibility.

### 12.5.1 Interaction with Biological Systems

When nanostructured devices interact with biological systems, they establish an extensive surface area for interaction. This large interface can lead to beneficial outcomes, such as enhanced cellular responses and improved therapeutic efficacy, or it may present toxicological challenges, including unintended immune reactions or cellular toxicity. The impact depends largely on the physicochemical properties of the nanostructure, such as its size, shape, surface charge, and material composition. Thus, careful design and surface modification of nanostructures are crucial to optimizing their biocompatibility and minimizing adverse effects during biomedical applications.

### 12.5.2 Biocompatibility Tests

According to ISO 10993, biocompatibility refers to the ability of a material to elicit an appropriate host response under specified conditions, emphasizing a beneficial interaction rather than simple inertness. For example, the microstructuring of titanium implant surfaces can modify how blood proteins adhere to the material, enhancing cell adhesion and integration [70].

Biological testing considerations [71]

1. Usage of medical device in final finished form or representative test article
2. Examination of in situ polymerizing and/or absorbable materials
3. Biological response resulting from mechanical dysfunction of the device
4. Submicron or nanotechnology components: Immunogenicity, toxicity, agglomeration, or aggregation

There are many phases of biocompatibility testing, and each one assesses a distinct facet of the material's interaction with biological systems:

1. Primary testing comprises *in vitro* investigations that evaluates the material's effects on blood, DNA, and cell viability. These tests include hemolysis, genotoxicity, and cytotoxicity assessments.
2. Secondary testing: Offers information on localized responses by evaluating the possibility for sensitization, irritation, and implantation effects on mucosal or cutaneous surfaces.
3. Advanced testing is done to investigate bigger repercussions on the organism, concentrates on carcinogenic potential, reproductive effects, and systemic toxicity.
4. Clinical evaluations: These comprise practical evaluation of the material's effectiveness in human participants.

Furthermore, in conformity with MD classification guidelines from regulatory bodies like the FDA and EMA, specific tests matches to the intended use of the device (e.g., stent expandability, porosity characteristics, and barrier functionality for devices like condoms) are carried out and correspond to the associated risk toward patient. These extensive testing procedures guarantee that a material is not only acceptable for its intended medical use but also biocompatible.

## 12.6 PHARMACOKINETIC CONSIDERATIONS

A protein corona is quickly developed around a nanomaterial in a biological environment, leading to a dynamic equilibrium. The material's nanoscale characteristics, such as its specific surface area, surface reactivity, and general structural features, have a significant effect on this equilibrium. Biological entities, such as proteins, cells, or tissues, have different affinities for different materials, which depends on whether they are in a bulk or nanometric state. Because of their greater abundance, proteins at higher concentrations first stick to the surface. These are progressively substituted by proteins that have a higher binding affinity to the nanomaterial while

being less concentrated. A stable yet dynamic protein layer is formed in part by this replacement mechanism. Protein adsorption is primarily identified by surface characteristics including charge, roughness, and hydrophobicity, but the composition of the nanomaterial itself is also very crucial. As an example, a structural rearrangement in core/corona nanoparticle systems may cause core components to migrate toward the surface, modifying the surface reactivity of the material [72, 73]. Additionally, functionalizing the surface with polymers like N,N-dimethylacrylamide (PDMA) or polyethylene oxide (PEO) can govern the protein adsorption. These polymers provide a steric barrier that limits protein binding when grafted onto materials like poly(lactic-co-glycolic) acid (PLGA) with PEO. This may alter the biological response and lessen unwanted interactions with the immune system [74, 75]. The development of biocompatible nanomaterials and their applications in medication administration and medical implants are dependent on this capacity to regulate protein adsorption.

### 12.6.1 Pharmacokinetic Features of Possible Degradation Products [76–78]

The pharmacokinetic aspects that are in relation to the degradation products emitted by the nanoparticles found in MDs are the primary subject of this section. Independent of how they were integrated at first; these materials degrade into a range of products, such as colloidal nanoparticles trapped in the matrix.

- Colloidal nanoparticles incorporated into the matrix;
- Dispersed fragments of nanoparticles;
- Metallic ions emitted due to the dissolution of nanoparticles or the breakdown of the metal-based device;
- Corrosion products developing on the outside of the device.

The distribution, metabolism, and clearance of these breakdown products by biological systems, which might have a possible impact on the safety and effectiveness of the nanomaterial. Assessing the biocompatibility and long-term impacts of nanoparticles in medical applications requires an understanding of these interactions.

The safety and efficacy of the nanomaterial may be impacted by changes in the distribution, metabolism, and clearance of these breakdown products by biological systems. Understanding these interactions is necessary to assess the long-term effects and biocompatibility of nanoparticles in medical applications.

## 12.7 ADVANTAGES OF NANOCOATING [79–81]

Nanocoatings principally increase the robustness, utility, and safety of MDs. Here are a few of the principal benefits.

1. Enhanced biocompatibility: By utilizing nanocoatings, MDs can have their biocompatibility improved, which reduces inflammation and immunological responses. Consequently, these devices are more suitable for use within the human body.

2. Enhanced antibacterial qualities: Nanocoatings, especially those comprising silver, zinc oxide, or copper nanoparticles, can deliver strong antimicrobial capabilities. This minimizes the possibility of infections from external or implanted medical equipment.
3. Reduction of biofilm formation: Biofilms can cause major infections and are famously difficult to treat. Nanocoatings can reduce the development of biofilms on MDs including stents, implants, and catheters by preventing bacteria from adhering to surfaces early.
4. Increased durability and toughness: Mechanical properties that includes durability, hardness, and resistance to wear, can be improved by application of nanocoatings onto it. This is especially advantageous for machinery that is subjected to a lot of mechanical stress, such as orthopedic implants.
5. Improved drug delivery: MDs that are prepared with functionalized nanocoatings can release drugs under regulated conditions. This can be utilized in implants, wound dressings, or stents to provide better mechanical properties as well as localized, enhanced cellular uptake, longer tissue residency, reduced local overdose, prolonged drug release at the preferred site.
6. Better hemocompatibility: By lowering thrombogenicity, or the propensity for clot formation, nanocoatings for blood-contacting devices like vascular stents make them safer to use in the cardiovascular system over an extended period of time.

## 12.8 REGULATORY CHALLENGES AND POTENTIAL RISKS AND ETHICAL CONSIDERATIONS

Nanomaterials in pharmaceuticals pose challenges due to their complex nature, beyond the scope of standard criteria typically used for their classification. Quality control of nanomaterials is more intricate compared to bulk materials, necessitating new or adapted analytical techniques to provide detailed characterization. Due to insufficient dedicated pharmaceutical quality control standards and regulatory guidelines creates uncertainty, as neither the United States nor European Pharmacopoeias have monographs specifically for nanomaterials. Variability in national definitions, despite ISO's international standards, further complicates regulatory consistency. A developing field of innovation is combination products that combine active chemicals, electronics, and nanostructures. But because different regional authorities may classify these goods differently as pharmaceuticals or MDs, they provide special regulatory issues. Harmonized frameworks are necessary because, in the absence of standard rules for nanomaterials, current good manufacturing practices (cGMP) must be adhered to. For example, the FDA and EMA jointly hosted a workshop in 2010 with the goal of standardizing regulatory procedures for nanomedicine. Nanomaterial manufacturing, storage, and degrading impurities introduce additional complexity that needs thorough characterization and standards. Addressing possible toxicity issues requires an understanding

of how nanoparticles interact with biological systems. Additionally, the emerging field of combination products those that integrate pharmaceuticals, biologics, and/or MDs could see the introduction of new regulatory categories to accommodate innovations like DES and transdermal patches. Despite the FDA's definition of a drug based on its primary mechanism, the licensing process for products combining drugs and nanomaterials remains complex. This complexity suggests a future where the regulatory distinctions between pharmaceuticals, nanomaterials, and MDs could become less defined, necessitating new approaches to their evaluation and oversight [71, 82].

## 12.9 SCALABILITY CONCERNS AND CHALLENGES FOR NANOCOATED MEDICAL DEVICES

Scalability concerns for MDs coated with nanoparticles include issues with material compatibility, coating process repeatability, and manufacturing technique optimization. For a device to be reliable, its coating qualities must be consistent throughout large production batches. It is necessary to do thorough testing for toxicity and interactions with biological systems in order to identify biocompatible nanomaterials that are compatible with the mechanical and chemical characteristics of the substrates. The optimization of high-throughput nanocoating techniques is necessary to maintain nanoscale functions while allowing for scaled manufacturing. Although it might require a lot of resources, accurate surface characterization using methods like AFM and SEM is essential for quality control. Comprehensive testing to prove safety and efficacy is necessary to navigate complicated regulatory procedures, yet doing so might present serious time and resource restrictions. Another issue is production cost, which might increase when using sophisticated manufacturing processes and obtaining high-grade nanomaterials. Under physiological circumstances, nanocoatings must remain stable for an extended period of time, as leaching or degradation might impair device functionality. It might be necessary to make major redesign and investment decisions in order to integrate nanocoating methods into current production systems. For patients and healthcare professionals to adopt the market, clinical advantages and safety must be shown. Additionally, in order to guarantee compliance and encourage sustainable practices in the marketing of nanocoated MDs, it is imperative to tackle environmental concerns and navigate intellectual property problems.

## 12.10 CHARACTERIZATION OF THE COATED DEVICES

MDs coated with nanoparticles are characterized using a range of analytical methods to assess their mechanical, chemical, biological, and physical characteristics. It is essential for the advancement of medical technology and better patient outcomes that these devices fulfill regulatory requirements and function as intended. Figure 12.7 illustrates the characterization techniques used for nanocoated devices.

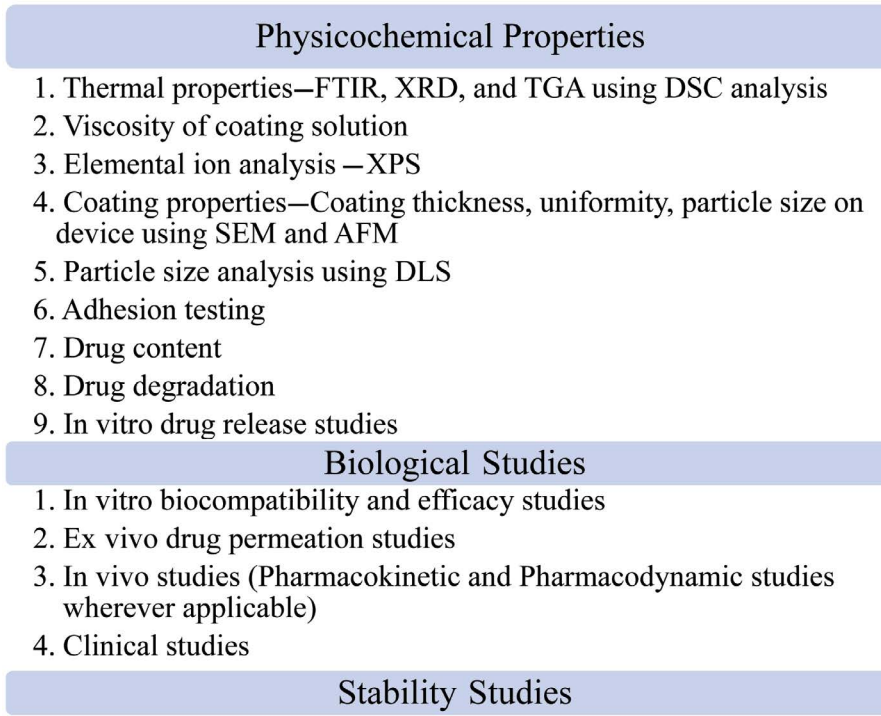


**FIGURE 12.7** Characterization techniques used for nanocoated devices.

## 12.11 MARKET AND AVAILABLE PRODUCTS FOR NANOCOATED DEVICES

The nanotechnology-based MD market, valued at over USD 3 billion in 2021, is projected to grow at a CAGR of over 8% from 2022 to 2030. This growth is driven by the technology's ability to enhance healthcare treatments and outcomes, particularly in wound care and implantable devices. By 2030, the implantable device segment is expected to exceed USD 2.9 billion. Key applications include orthopedics, which is set to grow at 8% CAGR due to nanotechnology's role in improving bone-implant integration through nanoscale scaffolds and materials. Tables 12.1 and 12.2 illustrate the clinical applications of nanocoated devices, along with several that are currently under investigation in clinical trials.

## 12.12 RECENT ADVANCES

In the last decade, the MD industry has experienced substantial research activity and rapid expansion. Moreover, advancements in micro- and nanotechnology now enable the manipulation of biological processes initiated upon implantation at sub-micron scales, allowing for targeted and effective interventions. Nanocarrier-based and nanostructured stent surfaces have significantly advanced cardiovascular treatment

**TABLE 12.1**
**List of Marketed Devices with Nanostructured or Nanocoated [83]**

| Organ | Medical Device | Name of the Marketed Device | Nanotechnology | Characteristics | Name of the Company (Country) |
|---|---|---|---|---|---|
| **Skin** | Bandage | Acticoat® | Silcryst™ | Silver nanocrystals (range from 1 to 100 nm) | Smith & Nephew (United Kingdom) |
| | Antimicrobial textile | Mipan® Magic Silver Nano | Silver NP | CAS number: 7440-22-4<br>Particle size <100 nm PVP stabilized –33.7 mV | Hyosung (Korea) |
| | Suture needle | Sandvik Bioline 1RK91™ | Steel nanocrystals | 1–10 nm | Ab Sandvik Materials Technology (Sweden) |
| **Eye** | Retinal implant | Argus™ | Nano-electronic component | N.A. | Second Sight Medical Products (USA) |
| | Retinal implant | N.A. | Silicium nanowires + iridium oxide nanolayer | 1 nanowire: 1 µm diameter and 9 µm height<br>Iridium oxide layer: 30 nm | Nanovision Biosciences / San Diego University |
| **Mouth** | Surgical mask | NanoMask® | Magnesium oxide and titanium dioxide NP | Positively and negatively charged NP | Nano Mask, Inc (USA) |
| | Surgical mask filtering influenza virus | NanoFense™ | Silver NP | N.A. | Applied Nanosciences |
| **Tooth** | Adhesive system | Adper™ Scotchbond™ SE | Silanized zirconium NP | 5-nm spherical particles under colloid form | 3M ESPE (USA) |
| | Dental restoration composite | Aerosil 200® | Silicium NP | Particle size 7 nm. Aggregates size 200–300 nm | EVONIK |
| | Dental restoration composite | Ketac™ N100 | Nano-ionomer | N.A. | 3M Espe (USA) |
| | Photopolymerisable dental restoration composite | Filtek™ Supreme | Silicium NP | N.A. | 3M Espe (USA) |
| | | Grandio® | Nano ceramic-hybrid composites | N.A. | Voco (Germany) |

*(Continued)*

**TABLE 12.1** ***(Continued)***
**List of Marketed Devices with Nanostructured or Nanocoated [83]**

| Organ | Medical Device | Name of the Marketed Device | Nanotechnology | Characteristics | Name of the Company (Country) |
|---|---|---|---|---|---|
| | Photopolymerisable dental restoration composite | Optiglaze | Silicium NP | N.A. | Gc Corporation (Japan) |
| | Dental restoration product based on synthetic resin | Kappalux Nano | Silicium and zirconium oxide NP | Particles size: 10–100 nm | Produits Dentaires Pierre Rolland (France) |
| | Metallic dental implant | NanoImplant® | Titanium NP | Particles size: 100–300 nm | Timplant (Czech Republic) |
| | Product for injectable bone filling | Nanogel® | Hydroxyapatite NP | Particles size: 100–200 nm | Teknimed (France) |
| **Bone** | Product for injectable bone filling | NanostimTM / Ostim® | Hydroxyapatite nanofibers | Nanofiber size: 100 nm length and 5 nm width | Aap Biomatierals (Germany) / Medtronic (France) |
| | Nanostructured metallic Orthopedic implant | | | Surface area/mass : 106 $m^2/g$ | |
| | | PerOssal® | Hydroxyapatite NP | N.A. | Aap biomaterials (Germany) |
| | | FortrOss® | Hydroxyapatite NP (NanOss®) combined to osteoconductive E- Matrix | N.A. | Pioneer Surgical Technology (USA) |
| | | Vitoss® Scaffold | Calcium phosphate NP | Particle size: 100 nm | Orthovita (USA) |
| | | Puretex® | Nanostructured titanium surface | N.A. | Sybron Implant Solutions (USA) |
| | Orthopedic or joint prosthesis | Nanos™ Symax™ | Microporous coating (Bonit®) with hydroxyapatite nanocrystals (made by DOT (Allemagne)) | N.A. | Smith & Nephew (United Kingdom) Stryker (France) |

*(Continued)*

**TABLE 12.1** ***(Continued)***
**List of Marketed Devices with Nanostructured or Nanocoated [83]**

| Organ | Medical Device | Name of the Marketed Device | Nanotechnology | Characteristics | Name of the Company (Country) |
|---|---|---|---|---|---|
| **Bone/ joint** | Orthopedic or teeth prosthesis | NanoTite™ | Calcium phosphate nanocrystals deposited on a surface | Nanocristal size: 20–100 nm | BIOMET 3i |
| | External knee prosthesis | Rheo Knee | Iron NP | Particles size: 100–1000 | Ossur (Iceland) |
| | Surgical device delivering data on custom orthopedic knee implantation | Mako | Nanosensors | N.A. | ORTHOSENSOR (USA) |
| **Vessel** | Stent coating | Debiostent™ | Nanoporous silicon membrane | Pores size: 1–250 nm<br>Membrane thickness: 50 nm–250 µm | DEBIOTECH (Switzerland) |
| | Naked coronary stent | Catania™ | Polyzene®-F | Coating thickness: 40–50 nm | Celonova Biosciences (Canada) |
| | Drug-eluting stent | VestaSync™ | Nanoporous hydroxyapatite coating | Coating thickness: 100 nm | Miv Therapeutics (Canada) |
| | Vascular graft | UCL-NanoBio™ | Polymer nanocages | Cage size: 200–500 nm<br>Cage height: 100–200 nm | Royal Free Hospital Of London (United Kingdom) |
| | Catheter for anesthesia | ON-Q® SilverSoaker™ | Coating including silver NP (SilvaGard™ made by ACRYMED (USA)) | Particles size: 10 nm | I-FLOW CORP. (USA) |
| **Abdomen** | Ventral Hernia Mesh | NovaMesh™ | Electrospun nanofibers integrated into a tissue | N.A. | Nicast (Israel) |

**TABLE 12.2**
**A List of Nanostructured Devices Currently Undergoing Evaluation in Clinical Trials [83]**

| Study Name Official Title ClinicalTrials.gov Identifier: | Medical Device Nanoparticles | Status | Purpose |
|---|---|---|---|
| IABN<br>Clinical Study: The Antibacterial Effect of Insoluble Antibacterial Nanoparticles (IABN) Incorporated in Dental Materials for Root Canal Treatment NCT01167985 | Dental restoration<br>Polyethyleneimine nanoparticles | Unknown | Effect of antibacterial nanoparticles, incorporated in root canal sealer material and in provisional restoration |
| Clinical Study: The Effect of Addition of Insoluble Antibacterial Nanoparticles (IABN) in Resin Base Provisional Cement NCT00502606 | | | Effect of antibacterial nanoparticles, incorporated in cement, on *S. mutans* in the margins of provisional restorations |
| Clinical Evaluation of Esthetic Restorations Placed in Primary Molars with Composite Resin Enriched with Insoluble Anti-bacterial Nanoparticles (IABN) NCT00389714 | | | Effect of antibacterial nanoparticles |
| Clinical Study of Antibacterial Nanoparticles Incorporated in Composite Restorations NCT00299598 | Alkylated polyethylenimine nanoparticles | Completed | Resin composites withholding antibacterial properties useful in preventing recurrent caries |
| Altrazeal Range of Motion Study Comparing with Typical Carboxymethyl (ROM) NCT01062191 | Hydrogel bandage Nanoflex® NP | Terminated due to a lack of enrollment | Difference in joint range of motion of a new flexible hydrogel nanoparticle wound dressing compared to typical sodium carboxymethylcellulose dressing Aquacel AG for treatment of partial-thickness burns |
| SNOOPY2 Exhaled Breath Olfactory Signature of Pulmonary Arterial Hypertension NCT02782026 | Electronic nose (E-nose)<br>Gold nanoparticles coated with organic ligands | Currently recruiting | Evaluate the diagnostic performance of a novel electronic nose (E-nose) for the detection of pulmonary arterial hypertension (PAH) |
| Wear Characteristics of Denture Teeth NCT01188226 | Denture teeth<br>Nano hybrid composite | Terminated | To evaluate the wear characteristics of new resin denture teeth (nano particles – hybrid composite) made by an injection technique |

by enhancing drug delivery and biocompatibility. Polymeric nanoparticles, especially those carrying anti-restenotic agents or paclitaxel, show improved arterial retention and uptake, with smaller particles performing better in targeting VSMCs. Modified coatings like cationic nanoparticles and sirolimus-loaded micelles enable deeper tissue penetration and reduced inflammation. Nanostructured surfaces, such as CNTs, metallic nanopillars, and nanoporous alumina, improve EC growth, biocompatibility, and reduce thrombogenicity. Innovations like gene-bearing coatings further enhance healing and prevent restenosis, making these approaches promising for cardiovascular applications [36, 84–91]. Recent advances in micro- and nanoengineered implants have significantly improved neural, retinal, and cochlear therapies. Polymeric/lipid-based implants like PLGA fibers and microparticles offer sustained drug release for glioma treatment, while advanced scaffolds help manage epilepsy and Parkinson's by maintaining neurotransmitter levels [53, 56, 92–95]. Neural/retinal/cochlear implants with coatings such as $Al_2O_3$, silicon-based 3D, and CNTs enhance biocompatibility, signal conduction, and neurite growth. Functionalized surfaces like PEDOT and diamond-based retinal prostheses support better cell adhesion and vision restoration. These technologies also enable targeted drug and gene delivery for inner ear conditions, paving the way for improved treatments in neurological and sensory disorders [61, 96–100].

## 12.13 CONCLUSION

Nanocoated MDs have had a substantial commercial impact on the MD industry, significantly enhancing the therapeutic effectiveness of these coated devices and, consequently, improving patient compliance. The incorporation of nanotechnology not only optimizes the performance of medical implants but also addresses critical issues such as drug delivery efficiency and biocompatibility, leading to better patient outcomes. However, the large-scale manufacturing of nanocoated devices presents notable challenges, particularly concerning regulatory compliance. The complexities associated with the standardization and validation of nanotechnology applications necessitate rigorous regulatory frameworks to ensure safety and efficacy. The lack of comprehensive regulatory guidance can hinder market entry and slow the adoption of innovative nanocoated solutions in clinical practice, underscoring the need for collaborative efforts between industry stakeholders and regulatory agencies to establish clear guidelines and facilitate the responsible development of these advanced medical technologies.

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